

Effects of Smoking on Lipid Content, Macromolecular Structure and Rheological Properties of *Hevea brasiliensis* Sheet Rubber

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ABSTRACT

Natural rubber ribbed smoked sheet (RSS) production process involves a drying step in a wood smoke dryer. The purpose of this work was to know whether this smoke drying step affected the lipid composition and the properties of sheet rubber. This study consisted of a comparative study between unsmoked sheets (USS) and ribbed smoked sheets (RSS) obtained from monoclonal latices from RRIM600, BPM24 and PB235 clones. The rubber was sampled in Chantaburi province, Thailand. It was found that the smoking process increased significantly the amount of lipid extract but decreased significantly the free fatty acid content. No significant effect of smoking was detected on macromolecular parameters such as gel content or molar mass distribution and on rheological parameters such as initial plasticity (P_0) and Mooney viscosity ($ML_{(1+4)100}$). In terms of resistance to thermal oxydation estimated by Plasticity retention index (PRI), smoked sheets displayed a tendency to have lower PRI than unsmoked sheets. This difference was significant for PB235 clone. A clonal effect was detected for most of the measured parameters. Indeed, PB235 clone displayed a higher lipid extract, higher P_0 , higher $ML_{(1+4)100}$, higher molar mass than those of the other studied clones while its PRI was lower.

Key words: *Hevea brasiliensis*, natural rubber, ribbed smoked sheets, smoking, rubber lipids

INTRODUCTION

Natural rubber (NR) is the most used elastomer and its share in world rubber consumption has been stabilized around 40% for more than 15 years (Samosorn, 2007). It has been used for over a century for end-user products mainly for the tire sector, but remains described as an enigmatic material with unequalled properties such as strain-induced crystallization,

tensile strength, high resilience, resistance to impact and tear, low heat build-up, good green strength, and building tack. Even if its synthetic counterparts have been commercially available for more than 50 years, NR appears to be a practically irreplaceable elastomer.

In Thailand, NR is a major agricultural commodity. Thailand is the first exporter in the world, with almost 3 million tons in 2006 which was 4.2% of the total value of Thai

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exportation. The Thai NR is exported as block rubber (STR) for 38%, Ribbed Smoked Sheets (RSS) for 32%, and concentrated latex for 30% (Samosorn, 2007).

The recognized quality of sheet rubber comes essentially from their very low level of impurities due to a clean process and a systematic visual quality control. Smoking rubber sheets is a well-known strategy for preventing them from mould growth and for decreasing water content. It was already described in 1935 to be an "old process" used as an attempt to imitate the smoke drying of thin films of latex in the preparation of "Fine Hard Para" (Hasting, 1935). Few data are available concerning the effect of smoking on the properties of the obtained raw dry rubber. Hasting (1935) showed that ageing properties of smoked sheet vulcanizates were better than air dried sheets but no information was provided about raw rubber properties. Bristow and Sears (1998) did a comprehensive comparative work on sheet and crumb rubber; unfortunately, the latex was stabilized for constant viscosity by the addition of hydroxylamine neutral sulphate which leaded to specialty products different from RSS. A recent study on the sheet rubber process proposed to replace the usual acetic or formic acid in the coagulation process by "smoke acid" extracted from smoke (Ferreira, 2005); however, no comparison with unsmoked sheets was established.

The recognized properties of natural rubber are mainly due to the presence in *Hevea brasiliensis* latex of a very long chain polymer of *cis*-1,4-isoprene but it is believed that the irreplaceable character of its properties is linked to its non-isoprene components, which represent 4 to 5% of latex weight (D'Auzac, 1989). Most of the latex non-isoprene components are hydrophilic molecules such as proteins and carbohydrates that could be leached during the process leading to dry rubber. Some other compounds, such as lipids, are retained in the dry rubber and have been found to

affect rubber properties (Hasma, 1984; Visitnonthachai, 2005). For instance, fatty acids were found to have an effect on the crystallization and plasticizing of rubber. The rate of crystallization of natural rubber is affected by the nature of the long-chain free fatty acids as well as by the presence of fatty acids esterified to the α -terminal of the polyisoprene chain. Rubber miscible fatty acids (unsaturated) and immiscible fatty acids (saturated) have been shown to stimulate synergistically the rate of crystallization, which explained the specific green strength of natural rubber (Tanaka, 1997). The influence of lipids on vulcanization characteristics (scorch and cure times) and vulcanizate properties (tack, tensile and tear strengths) have been also pointed out in recent studies (David, 2000; Ismail, 2001). It is therefore interesting to follow lipid composition, and especially free fatty acids, when conducting studies on natural rubber quality.

The purpose of this study was to know whether the smoke drying step of the post-harvest process affects the lipid composition and the properties of sheet rubber. The present work consisted of a comparative study between unsmoked sheet (USS) and ribbed smoked sheet (RSS) obtained from monoclonal latices.

MATERIALS AND METHODS

Material

Natural rubber

Hevea brasiliensis clones RRIM 600, PB 235 and BPM 24 were used in this study. The samplings were performed in Union Rubber Co. Ltd. Chantaburi, Thailand on fields 93, 100 and 111 for RRIM 600, PB 235, and BPM 24 clones, respectively. Four sampling repetitions were performed on 20 January 2006, 28 March 2006, 7 July 2006 and 24 October 2006.

The following chemicals used in this study were of analytical grade: acetic acid (Merck, Germany), chloroform (Labscan, Thailand),

methanol (Merck, German), *n*-hexane (BDH, England), sodium chloride (APS Finechem, Australia), rhodamine 6G (Fluka, UK), cyclohexane (Carlo Erba, Italy). Formic acid used for latex coagulation was of commercial grade.

Methods

Unsmoked sheet (USS) preparation

Unsmoked sheets were prepared as recommended by the Rubber Research Institute of Thailand (RRIT). About 3 L of monoclonal latex were filtered (pore size 2 mm). The filtered latex was added with 2 L of water. The diluted latex was coagulated into a coagulation tank (35×20×9cm) with addition of 300 mL of 2 % formic acid solution (obtained by dilution by 45 times of commercial grade formic acid). The obtained coagulum was pressed with a crusher (one pass), a flat roll hand mangle (3 passes) and a rough roll hand mangle (2 times). The obtained fresh rubber sheet was washed with water several times and dried outside under the shade until full disappearance of white spots.

Rubber smoked sheet (RSS) preparation

Fresh rubber sheets were prepared as described above. They were then subjected to smoke drying in Chachoengsao Rubber Research Center (CRRC). The drying conditions were as follows: fresh sheets were hanged on a special trolley which was placed for 3 days in a smoke chamber (3×10×3m) where the temperature was regulated to 50 °C. The smoke was generated by burning about 1m³ of wood for the 3 days smoking period.

Lipid analysis

Extraction

A piece of 2.8 grams was sampled from rubber sheets (USS or RSS) and cut into small pieces (2×1×1 mm). The obtained pieces were stored at -18°C overnight. Grinding of the frozen pieces was performed using a cryogrinder

Pulverisette 0 (Fritsch, Germany) under liquid nitrogen with 2 mm amplitude for 4 min. The obtained ground rubber (diameter of around 0.2 mm) was transferred directly after grinding into 50 mL of a mix of chloroform:methanol (2:1 v:v). After agitation at 160 rpm on a rotary agitator for 6 h at room temperature, extracted rubber was removed by filtration and the total extract was concentrated in a rotary evaporator. Total extract was redissolved with 4 mL of chloroform:methanol (2:1 v:v) and the water soluble components were removed by washing with 1 mL of 0.9% NaCl solution following Folch procedure (Folch, 1957). The mixture was allowed to separate clearly and the lipid containing bottom layer was evaporated to obtain the lipid extract. The dry lipid extract was calculated versus initial weight of rubber.

Free fatty acids in lipid extract

The method described by Van Autryve *et al.* (1991) was followed. The analysis was based on the complexation of free fatty acid with Rhodamine 6G, which led to a specific absorbance at a wavelength of 513 nm. Calibration curves were obtained using linoleic acid in a range of 0 to 10 µg.mL⁻¹ solution in *n*-hexane. The lipid extract from rubber sheet was diluted to 20 µg.mL⁻¹ solution in *n*-hexane. A volume of 3.5 mL of diluted sample was added with 0.5 mL of the rhodamine 6G solution and the absorbance at 513 nm was read 5 min after mixing using a Hitachi U-2001 spectrophotometer (Tokyo, Japan). Free fatty acid content versus initial dry rubber was calculated using the calibration parameters.

Steric exclusion chromatography

Gel, number-average molar mass (M_n), weight-average molar mass (M_w) and molar mass distribution (MMD) of NR were characterized by size exclusion chromatography (SEC) following the method described by Bonfils *et al.* (2005). The

samples of NR were dissolved in cyclohexane at a concentration of 2 mg.mL⁻¹ in a total volume of 30 mL. After 14 days at 30°C with periodic agitation (1 h per day), insoluble part (macrogel) was removed by centrifugation at 16500 rpm for 1 h. The obtained solution was diluted to 0.2 mg.mL⁻¹ then further filtered (1 µm pore size) to remove microgel. Filtered solution (100 µL) was injected in a steric exclusion chromatographic system consisting of a ERMA ERC-3112 solvent gas remover, a Waters 510 pump, an automatic injector, a Waters 486 UV detector (220 nm), and two 30 cm PLGEL mixed columns (Polymer Laboratories) with a porosity of 20 µm. System temperature was regulated to 65°C and the flow rate of cyclohexane was 0.8 mL·min⁻¹. The entire installation was computer controlled using a dedicated software (Maxima-Waters). Calibration was performed by injecting polyisoprene standards in the range of 1 to 1200 kg.mol⁻¹. Microgel quantity was estimated by difference between the quantity of rubber obtained by rubber peak integration and the initial concentration before filtering (0.2 mg.mL⁻¹). Total gel was calculated by the addition of macrogel and microgel amounts.

Rheological properties

USS and RSS samples were sent to the Rubber technology division of RRIT for determining Initial plasticity (P_0), Plasticity Retention Index (PRI) and Mooney Viscosity $ML_{(1+4)100^\circ C}$ following Standard Malaysian rubber (SMR) standard bulletin n°7 (1992).

Statistical analyses

Statistical analyses (one way analysis of variance) were performed on the four replications using JMP software v.5.1.2. (SAS Institute, USA). Standard error (SE) bars and SE values are mentioned in figures and table, respectively. Significance level α of statistical analysis was set to 0.05.

RESULTS

Lipids

Lipid extraction results are presented in Figure 1. The lipid content of the unsmoked control was dependent on the clone: 2.25% (SE=0.03) lipids in dry rubber for RRIM600, 2.54% (SE=0.12) for BPM 24 and 3.13% (SE=0.09) for PB235 *Hevea* clones. Smoking significantly increased the amount of lipid extract to 2.59% (SE=0.04), 3.05% (SE=0.08) and 3.47% (SE=0.14) for the 3 clones, respectively. As shown on Figure 2, smoking reduced significantly the amount of free fatty acid from 0.8% to 0.5% versus dry rubber.

Macromolecular structure

No significant difference was observed on gel content as shown on Figure 3. Concerning the MMD of the remaining soluble part, no effect of smoking was observed either for rubber with a unimodal MMD with a shoulder (clone PB235) or for rubber with bimodal MMD (clones RRIM600 or BPM 24). An example of MMD is provided on Figure 7. All repetitions showed the same trend. Number-average molar mass (M_n), weight-average molar mass (M_w) confirmed that there is no difference (Table 1).

Rheological properties

Initial plasticity (P_0) and Mooney Viscosity ($ML_{(1+4)100}$) are presented on Figures 4 and 5, respectively. No significant effect of smoking was observed. When comparing by clone, it was to be noted that initial plasticity P_0 and Mooney viscosity were both significantly higher for rubber from clone PB235 than for rubber from the two other tested clones.

Resistance to thermal oxidation

No significant effect of smoking was observed on the plasticity retention index (PRI) for clones RRIM600 and BPM24. However, a

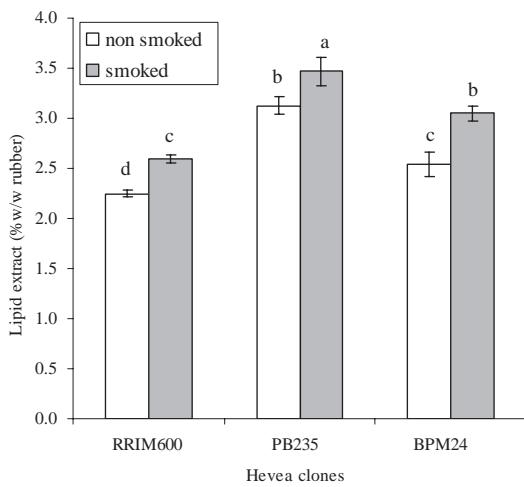


Figure 1 Lipid extract (% w/w rubber) by clone and treatment.

Error bar indicates standard error. Mean(s) associated with the same letter were not significantly different ($P \leq 0.05$).

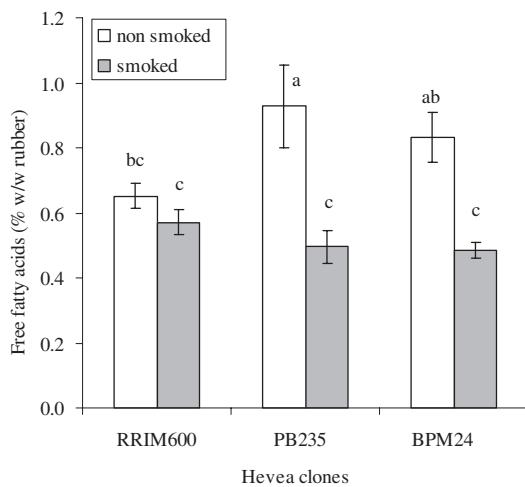


Figure 2 Free fatty acids (% w/w rubber) by clone and treatment.

Error bar indicates standard error. Mean(s) associated with the same letter were not significantly different ($P \leq 0.05$).

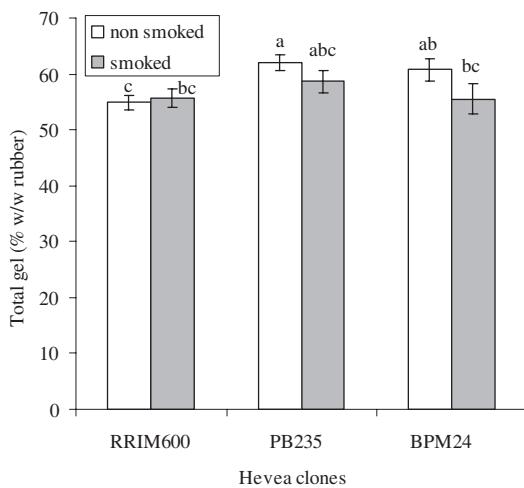


Figure 3 Total gel (% w/w rubber) by clone and treatment.

Error bar indicates standard error. Mean(s) associated with the same letter were not significantly different ($P \leq 0.05$).

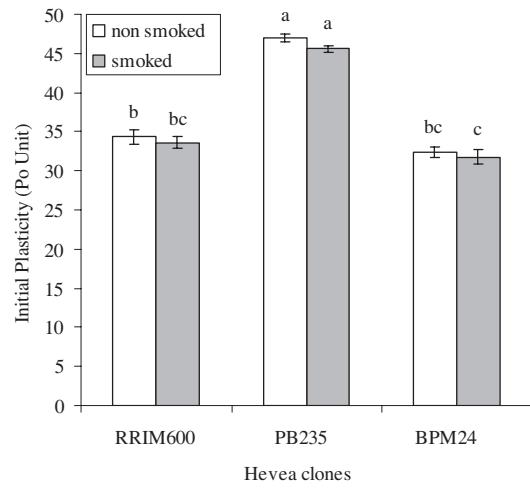


Figure 4 Initial plasticity (P_0 unit) by clone and treatment.

Error bar indicates standard error. Mean(s) associated with the same letter were not significantly different ($P \leq 0.05$).

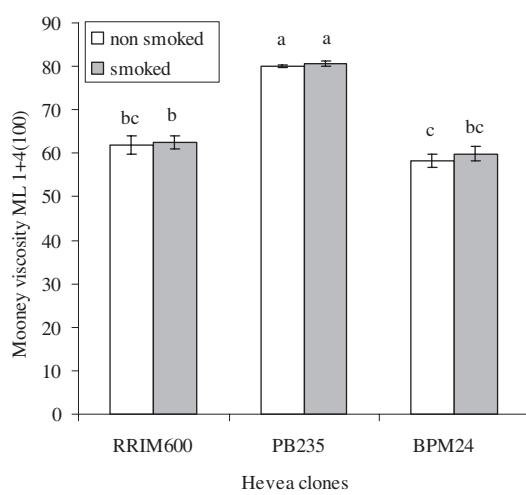


Figure 5 Mooney viscosity (ML₍₁₊₄₎₁₀₀) by clone and treatment.

Error bar indicates standard error. Mean(s) associated with the same letter were not significantly different ($P \leq 0.05$).

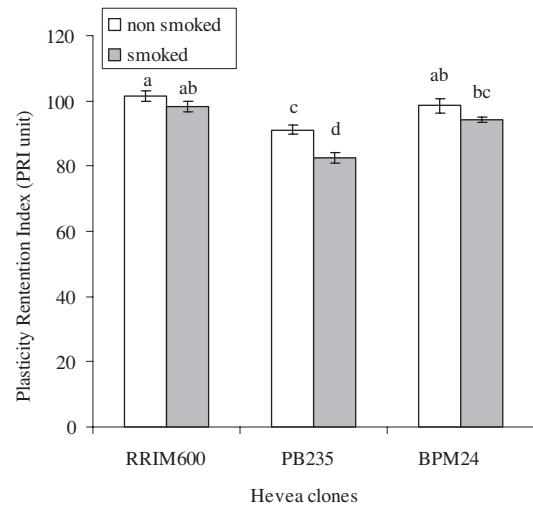


Figure 6 Plasticity Retention Index (PRI unit) by clone and treatment.

Error bar indicates standard error. Mean(s) associated with the same letter were not significantly different ($P \leq 0.05$).

Table 1 Weight-average molar mass (M_w) and number-average molar mass (M_n) by treatment for each clone.

Clones	Treatment	M_w (kg/mole)		M_n (kg/mole)	
		Mean	SE	Mean	SE
RRIM 600	USS	1285 ^{ab}	41.3	149 ^b	5.2
	RSS	1229 ^b	31.7	152 ^b	4.9
PB235	USS	1369 ^a	38.3	234 ^a	9.0
	RSS	1353 ^a	23.9	237 ^a	7.2
BPM24	USS	1237 ^b	54.8	144 ^b	7.8
	RSS	1095 ^c	41.2	139 ^b	4.9

For each column, mean values followed by the same letter were not significantly different ($P \leq 0.05$)

SE: standard error, USS: unsmoked sheet treatment, RSS: ribbed smoked sheet treatment

slightly negative effect (decrease of PRI by 8 points) was measured on PB235 rubber (Figure 6). This result was confirmed by a double analysis performed in France.

DISCUSSION

The two compared rubber sheet types, namely RSS and USS, differed from each other

by the drying process used. The two processes could indeed be distinguished by two factors: physicochemical conditions and duration of drying. In the case of smoked sheet (RSS) the process was performed over a period of 3 days, under smoke atmosphere and with a temperature of 50°C while unsmoked sheet (USS) was dried outdoor under shade over a period of approximately 8 days with temperature ranging

from 25 to 38°C.

The decrease of free fatty acid content may be explained by a protective effect of smoking: a faster drying and the presence of antimicrobial components in smoke, such as acetic acid or phenolic compounds (Holley, 2005), may prevent the degradation of native lipids by microbial hydrolases such as lipases or phospholipases. In the case of USS, lipid hydrolysis may be more important which results in less lipid extract containing more free fatty acids.

Nevertheless, rheological analyses such as Wallace initial plasticity or Mooney viscosity determination showed that the properties of the obtained rubber sheets were not changed whether a smoking step was present or not. This was confirmed by macromolecular structural results provided by steric exclusion chromatography that showed that poly(*cis*-1,4-isoprene) chain length was not affected by the smoking treatment.

Concerning resistance to thermal oxidation, a significant negative effect of smoking was observed for one of the tested clones, namely PB235, although smoke was supposed to contain antioxidant compounds. Even in the USS form, PB235 rubber seemed to be less protected against thermal oxidation as its PRI (91) was significantly lower than PRI of the two other clones (around 100). This clonal difference may be explained by a weaker reticulation process that occurs simultaneously with chain scission during aging in PRI oven. Nevertheless, all rubber sheets tested, smoked or not, were in an excellent range of PRI (>80).

In addition, this study confirmed that lipid content and rubber properties are strongly clone dependant. In our study, the rubber from *Hevea brasiliensis* PB235 displayed a significantly higher molar mass (expressed either with number-average (M_n) or with weight-average (M_w)), leading to higher plasticity and Mooney viscosity. This clone displayed also lower resistance to thermal oxidation and higher lipid content.

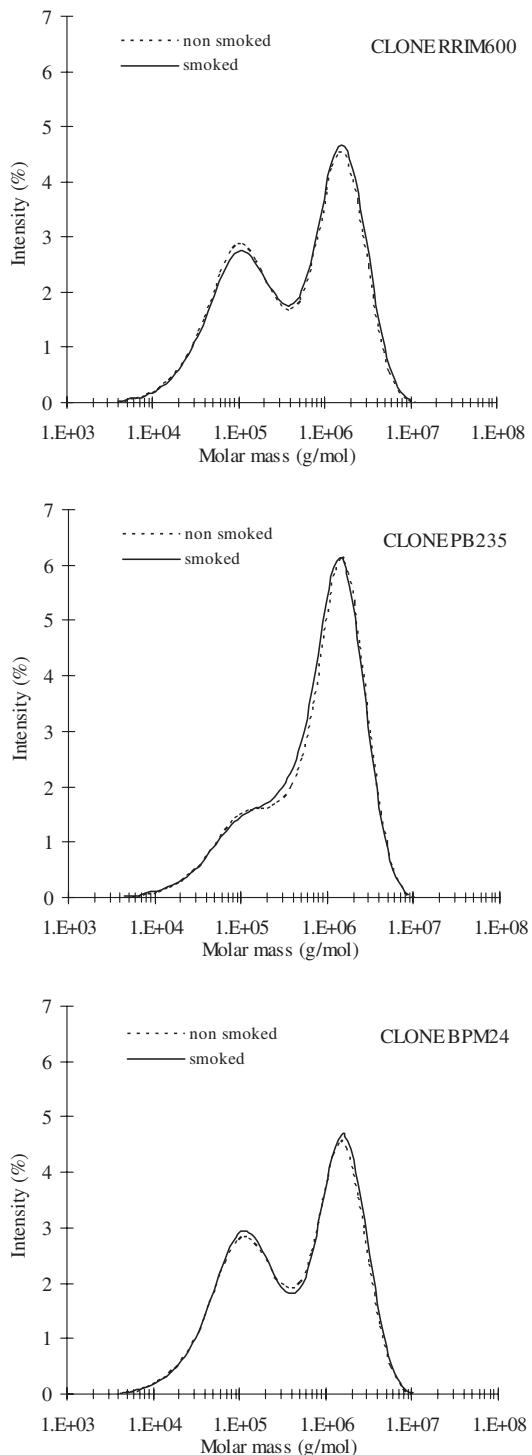


Figure 7 Molar mass distributions of rubber sheets determined by size exclusion chromatography (from sampling performed on 20/01/2006).

CONCLUSION

It was shown that the smoking process reduced the amount of free fatty acid in natural rubber sheets and increased the extractable lipids. No significant effect was observed on the macromolecular structure observed by gel quantification, molar mass distribution, and averaged molar masses. The rheological properties (P_0 , $ML_{(1+4)100}$) were not affected while a slight decrease of plasticity retention index was observed with rubber from PB235 clone.

Smoking thus seemed to preserve the lipid content of rubber but had no significant effect on physical properties of the dry product. It would be interesting to study separately the effect of the two main parameters involved in smoke drying when comparing with air drying: the duration of drying process (which was much shorter in smoking process) and the chemical effect of smoke content.

This study also showed that USS could be used as a representative sample of Thai rubber sheets even it was not the one that is used for exportation.

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