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Structural Characterisation of Wild Rubbers: Protein and Ester Content

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The latices of six species of wild rubber trees from the Apocynaceae *(1),* Euphorbiaceae *(3) and* Moraceae *(2) families were characterised in terms of rubber-bound protein and rubber-bound ester. FTIR and elemental analysis showed the presence of both groups and the protein seems to differ in type depending on the botanical family. The ester content was fairly constant in the same family but the type of ester present needs a more detailed study.*

Natural rubber (NR) contains about 6% of non-rubber components such as proteins, lipids and carbohydrates. Rubber hydrocarbon is reported to be composed of isoprene units, almost 100% in the *cis*-configuration. The non-rubber compounds are presumed to be responsible for the characteristic properties of NR, although there is no definitive structural information which interprets the origin of the outstanding properties of NR[1].

Recent studies indicate that the natural rubber produced by large-sized species (*Hevea brasiliensis* Muell. Arg.) has both soluble proteins in the latex as well as proteins linked to the polyisoprene[2] which can be almost completely removed by deproteinisation with an alkaline protease[3,4]. Besides, it was found that NR contains long-chain saturated and unsaturated fatty acids and their esters of about 15mmol/kg rubber. About one third of these fatty acid groups remained even after extraction with acetone, which were liberated as methyl esters by transesterification of NR with sodium methoxide[1,4].

With the objective of obtaining a better understanding about NR structure and verifying it among different species, three different botanical families, namely *Apocynaceae* (one *Hancornia*), *Euphorbiaceae* (three *Hevea*) and *Moraceae* (two *Ficus*) were studied.

The *Hancornia* trees ('mangabeiras') belong to a plantation with 80 copies placed in the Experimental Farm of Teaching and Research of the Faculty of UNESP-Ilha Solteira (FEP/FEIS) in the State of São Paulo. At the time of the collection of latex (February 1997) the trees were 10 years' old and approached a height of 6 m. *Hevea* trees were isolated specimens that had never been tapped and were placed in the Botanical Garden of Rio de Janeiro. *Hevea brasiliensis* (Muell. Arg.)

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var. *randiana* Pax is a tree of approximately 30 m of height, planted in 1917 and the other two species were more recent [*Hevea guyanensis* (H.B.K.) Muell. Arg. and *Hevea pauciflora* (Muell. Arg.) var. *coryaceae* Duke], which were at the most 10 m. The *Ficus* trees were also isolated and untapped species, found in FEP/FEIS with a medium age of 20 year's old and a height of around 10 m.

EXPERIMENTAL

Latex conserved in 7% ammonia was precipitated in acetone to obtain clots (HANR) that were cut in small pieces and extracted with acetone for 24 h using Soxhlet apparatus under nitrogen atmosphere. The preparation of deproteinised rubber (DPNR), transesterified rubber (TEDPNR) and the fractionation of the resulting rubbers by molecular weight followed a procedure from the literature[5]. All samples were extracted with acetone before analysis.

FTIR was obtained in a JASCO-5300 equipment with KBr cast films, resolution of 4 cm^{-1} and 500 scans.

$\overline{M}_n$ was obtained by size exclusion chromatography in a GPC-LALLS equipment with Jasco pump PU980 and TOSO detector LS8000 and two columns of poly(styrene-divinylbenzene) in series with exclusion limits of 2×10^7 and 5×10^4 g/mole.

The nitrogen content in the samples was obtained by elemental analysis in a YANCO MT-5 analyser using antipyrine as standard and a Mettler UMT 2 micro-balance to weigh the samples.

The ester content was obtained by FTIR[6] with the ratio between the 1738 cm^{-1} and 1664 cm^{-1} absorption bands related directly to the content of ester groups by the equation:

$$\text{Ester content (mmol/kg rubber)} = 157.8\left(\frac{A_{1739}}{A_{1664}}\right) - 1.4 \quad \ldots 1$$

RESULT AND DISCUSSION

Table 1 shows the dry rubber content obtained from the precipitation of latices, before and after the extraction with acetone. The values obtained with the mangabeira and *Hevea* rubbers are in agreement with the data in literature[8] and of all them, only the *Ficus* trees present a high content of non-polymeric compounds associated to the clot.

In *Table 2* it is observed that the *Hancornia* and *Ficus* samples possess the largest values of molecular weight although their dry rubber content was lower than in *Hevea* samples.

Nitrogen Content

The elemental analysis (*Table 3*) give the nitrogen content in the rubber hydrocarbon. The nitrogen content, which is presumed to be related to the presence of a protein linked to the ω terminal group of the polyisoprene[4], is low in the mangabeira rubber. By comparing their FTIR spectra of the HA and DP samples in the infrared region between 3000 cm^{-1} and 3500 cm^{-1} (*Figures 1a* and *1b*), it can be observed that the protein associated with the polymer in the sample HA is probably the oligopeptide type but it cannot be discarded as the possibility of oxidation of the terminal group in the polymer, releasing the polypeptide (which is extracted with acetone) and resulting in a low nitrogen content in the HA samples. The observation of the FTIR spectra of the *Ficus* rubbers in the same region, leads us to the same conclusion (*Figure 2*). It is relevant to note that natural rubber latex coming from different families have different stability towards oxidation[7].

TABLE 1. DRY RUBBER CONTENT IN THE LATEX OF *HANCORNIA*, *HEVEA* AND *FICUS* SPECIES

Specimen	Total solids (W%)	Dry rubber before extraction (% w/v)	Dry rubber after extraction (% w/v)	Rubber content in the clot (%)
Hancornia speciosa Gomez	45.2	31	No change	~100
Hevea guyanensis (H.B.K.) Muell. Arg.	52.6	38	No change	~100
Hevea pauciflora (Muell. Arg.) var. *coryaceae* Duke	56.3	44	No change	~100
Hevea brasiliensis (Muell. Arg.) var. *randiana* Pax	58.8	45	No change	~100
Ficus 1	35.1	10	5.0	34
Ficus 2	31.9	12	5.2	43

TABLE 2. MOLECULAR WEIGHT AND POLYDISPERSION IN THE RUBBER OF *HANCORNIA*, *HEVEA* AND *FICUS* SPECIES

Specimen	$\overline{M}_n \times 10^{-5}$ (g/mole)	$\overline{M}_w \times 10^{-5}$ (g/mole)	PD[a]
Hancornia speciosa Gomez	2.00	12.0	6.0
Hevea guyanensis (H.B.K.) Muell. Arg.	1.50	8.00	5.4
Hevea pauciflora (Muell. Arg.) var. *coryaceae* Duke	1.30	10.0	8.1
Hevea brasiliensis (Muell. Arg.) var. *randiana* Pax	1.30	5.20	4.1
Ficus 1	5.00	10.0	2.5
Ficus 2	2.20	7.70	3.5

[a]$PD = \overline{M}_w / \overline{M}_n$

Hevea rubbers possess a larger amount of nitrogen as it can be seen in *Figures 1c* and *1d* by the difference in the intensities between the two spectra (HANR and DPNR). This indicates a more complex peptide structure. The nitrogen contents obtained with *Hevea* rubbers are in agreement with previous data[1].

The low content of nitrogen obtained in some DPNR samples (*Table 3*) indicates the

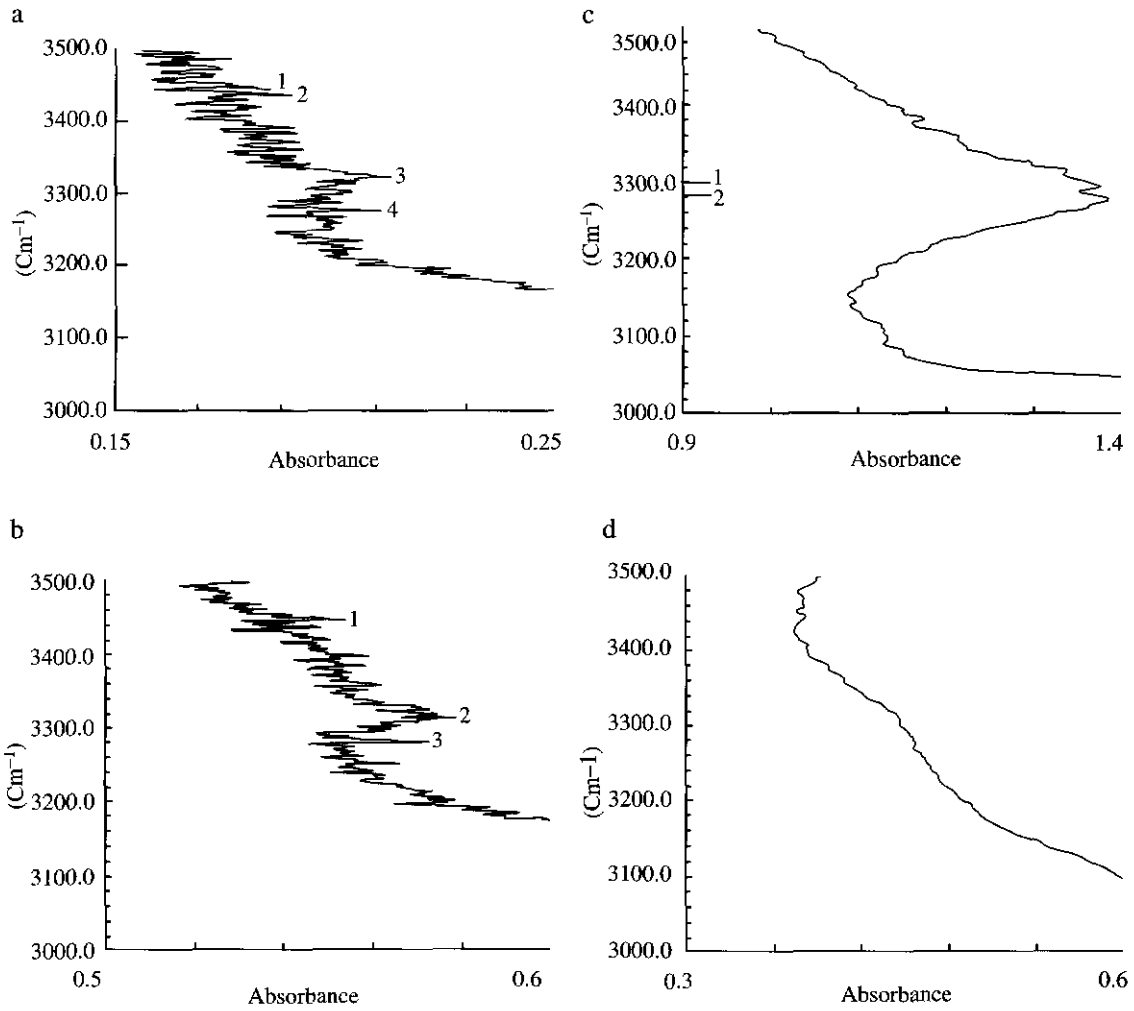

Figure 1. FTIR spectra of HANR and DPNR rubbers of Hancornia speciosa *Gomez (a and b) and* Hevea guyanensis *(H.B.K.) Muell. Arg. (c and d) in the region between 3000 cm^{-1} and 3500 cm^{-1}.*

presence of some residual nitrogenous components even after deproteinisation, probably amino-acids bound directly to the polyisoprene molecule. These results were found in other works[2–4]. In spite of the lack of nitrogen content data from *Ficus* rubbers, their spectra show some residual nitrogenous components in the sample of DPNR rubber by the absorption at 3319 cm^{-1} (*Figure 2b*).

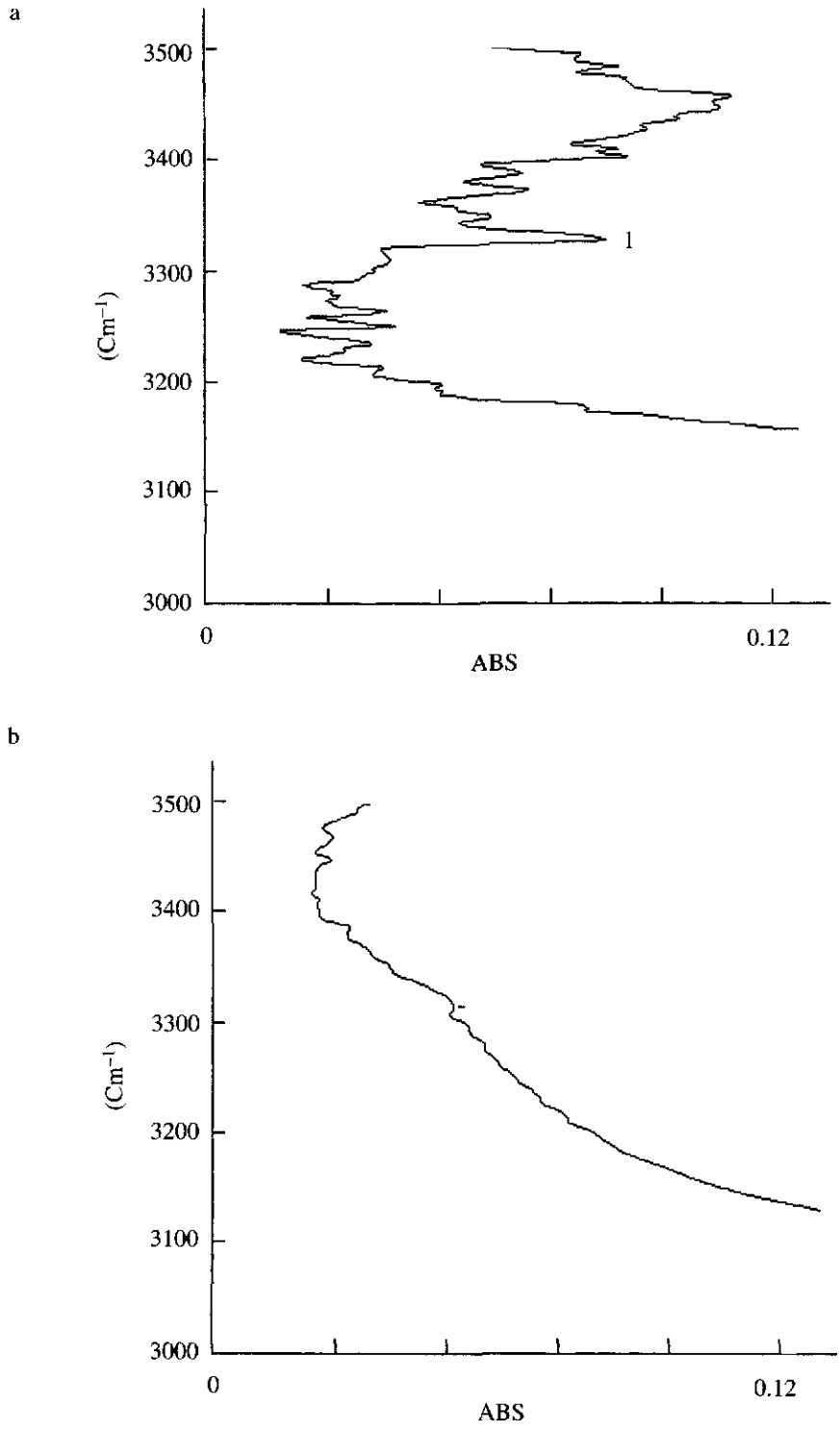

Figure 2. FTIR spectra of HANR (a) and DPNR (b) rubbers of Ficus *sp. rubber between the region of 3000 cm^{-1} and 3500 cm^{-1}.*

The Amount of Ester Groups

The amount of ester groups, obtained by the ratio between the 1734 cm^{-1} (C=O) and 1663 cm^{-1} (olefin) intensity bands show a good correlation with the HANR and DPNR samples. The difference between the HANR and DPNR ester values for each sample was around 10%. This is reasonable, because the enzymatic deproteinisation decomposed only peptide linkages.

Table 3 shows the content of ester groups present in the samples as an average between the HANR and DPNR ester values for each sample. The lowest value belongs to mangabeira rubber (5.3 mmole/kg rubber), the higher to *Ficus* rubbers (16 mmole/kg rubber) and the *Hevea* rubbers show an average value of 8.5 mmole/kg of rubber.

The non-rubber compounds isolated from the solution after transesterification of the samples were subjected to acetone extraction, neutralisation with diluted HCl, evaporation to dryness and drying *in vacuo* to give a constant weight and followed by extraction with acetone. The soluble product in acetone was analysed by FTIR and gas chromatography. The FTIR spectra of the extracts of the transesterification show the absorption bands at 1738 cm^{-1}, characteristic of carbonyl groups and a similar profile among them. The chromatograms of the samples present a complex pattern indicating besides some impurities, the presence of several fatty acid methyl esters with different chain lengths. The analysis of these fatty acids is in progress.

CONCLUSIONS

Data from FTIR obtained with deproteinised and transesterified rubbers show the presence of bounded proteins and fatty acids to the rubber chain. The protein content in the *Hevea* family is high compared with the other samples but data are not sufficient to distinguish different proteins among botanical families. The amount of ester is different depending on the botanical family and seems to be regular in samples of the same family.

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TABLE 3. NITROGEN AND ESTER CONTENT IN THE RUBBERS OF *HANCORNIA*, *HEVEA* AND *FICUS* SPECIES

Item	*Hancornia speciosa* Gomez	*Hevea guyanensis* (H.B.K.) Muell. Arg.	*Hevea pauciflora* (Muell. Arg.) var. *coryaceae* Duke	*Hevea brasiliensis* (Muell. Arg.) var. *randiana* Pax	*Ficus* 1	*Ficus* 2
Nitrogen (HANR)	0.116	0.488	–	0.350	–	–
Content (w%) (DPNR)	0.021	0.043	<0.001	<0.001	–	–
Ester content[a] (mmole/kg of rubber)	5	9	10	7	16	16

[a]Data are an average between HANR and DPNR samples

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