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NOTE

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Comparative study on the chemical composition of lipophilic fractions from three wood tissues of *Eucalyptus* species by gas chromatography-mass spectrometry analysis

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Abstract The chemical compositions of lipophilic fractions from *Eucalyptus urograndis* and *Eucalyptus urophylla* cultivated in Brazil and *Eucalyptus camaldulensis* from Mexico were determined by gas chromatography-mass spectrometry (GC-MS) before and after alkaline hydrolysis followed by derivatization. In all fractions, fatty acids (including small amounts of α- and ω-hydroxy fatty acids) and sterols were the most abundant components followed by smaller amounts of long-chain aliphatic alcohols, phenolic acids, and hydrocarbons. The presence of steroid esters and triacylglycerols in all three species was indirectly confirmed by the increased amount of fatty acids and sterols (manly β-sitosterol) in the hydrolyzed fractions compared with the corresponding nonhydrolyzed fractions. The amount of liphophilic compounds (mainly fatty acids and sterols) identified in hydrolyzed fractions of *E. urograndis*, *E. camaldulensis*, and *E. urophylla* corresponded to 1921, 1915, and 634 mg kg^{-1} of dry matter, respectively. The lower abundance of fatty acids and sterols in the fractions from *E. urophylla* indicates that problems related to pitch formation will be less severe for this species than for the other two.

Key words Lipophilic extract · Pitch · *Eucalyptus camaldulensis* · *Eucalyptus urograndis* · *Eucalyptus urophylla*

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Introduction

Hardwood species are presently the most important source of wood for pulp production. European white birch (*Betula pendula*) is the dominant species in North European countries whereas *Eucalyptus* species (mainly *Eucalyptus globulus*, *Eucalyptus grandis*, and *Eucalyptus urograndis*) are dominant on the Iberian Peninsula and in South America.[1] The increasing interest in wood from *Eucalyptus* species is due to their rapid growth and their interesting properties in terms of pulping, bleaching behavior, and final pulp quality.[2–5]

In recent years, the study of the composition and behavior of *Eucalyptus* wood tissues has been mainly focused on *E. globulus*, the dominant species cultivated on the Iberian Peninsula. Recently, the major features of *E. globulus* wood extractives[4] and macromolecular components[5] have been reviewed. Among *E. globulus* components, the lipophilic extractives fraction has been discussed in a significant number of publications[6–15] due to its impact on the formation of pitch deposits,[10,16,17] and also because these components might contribute to the consumption of bleaching chemicals.[4,5,18–20] For *E. globulus*, this lipophilic fraction is mainly composed of fatty acids and alcohols, fatty acid esters, triacylglycerols, hydrocarbons, and sterols.[6–14,19]

Although the information gathered for *E. globulus* might be relevant to design strategies to prevent pitch episodes in bleached pulp mills operating with other *Eucalyptus* species, it is well known that for the *Eucalyptus* genus there are strong variations in the chemical composition among and within species and with geographic location.[11,13]

The growing interest in the use of *Eucalyptus* for pulp production in Brazil has promoted research activities in the clone and genetic selection, and is aiming to improve the quality and productivity of Brazilian *Eucalyptus* forests.[21–23] In this perspective, detailed knowledge of the chemical composition of the lipophilic fraction of wood extractives from the *Eucalyptus* species cultivated in Brazil (*E. grandis*, *E. urophylla*, and *E. urograndis*) will be an important con-

tribution to improving industrial strategies for the prevention of pitch deposition during pulp bleaching. However, despite the high importance of these *Eucalyptus* species in the Brazilian pulp industry, as well as the importance of the Brazilian pulp and paper industry for the worldwide market, to the best of our knowledge, very little research has been carried out on the chemical composition of the lipophilic extractives of these species from plantations in Brazil.[19,24–26]

Within a wider project aiming to carry out a detailed study of the chemical composition of woods from *Eucalyptus* species cultivated in Brazil, in the present work we report the detailed chemical analysis of the lipophilic extractives from *E. urograndis*, *E. urophylla*, and *E. camaldulensis* before and after hydrolysis. This last species, although not largely cultivated in Brazil, is very important in the pulp and cellulose industry in Mexico and Thailand and has been considered in the present work for comparison.

Materials and methods

Samples

Eucalyptus urograndis and *Eucalyptus urophylla* wood samples were obtained from an 8-year-old plantation in Brazil. *Eucalyptus camaldulensis* wood (12-year-old trees) was obtained from a Mexican plantation. The wood material used was bark free, chopped into small pieces (industrial size), and air-dried at ambient temperature for 5 days. It was then ground to pass a 1-mm sieve screened in a vibratory sieving apparatus and the 40- to 60-mesh fractions were used for chemical analysis, according to described experimental procedures.[27]

Extraction

The air-dried powdered samples (2 g) were extracted with acetone for 6 h using a Soxhlet apparatus. The solvent was removed under reduced pressure in a rotary evaporator and the fractions were weighed. All extractions were carried out in triplicate and the extraction yields were expressed as percentages in relation to the wood's dry weight.

To isolate the lipophilic fraction, the acetone extract was redissolved in dichloromethane (3×2 ml) and filtered off. The derivatized dichloromethane-soluble (lipophilic) residues were analyzed by gas chromatography-mass spectrometry (GC-MS) before and after hydrolysis as described below.

Alkaline hydrolysis

To a two-necked round-bottomed flask (10 ml) was added 10 mg of the dichloromethane extract, followed by 1.8 ml of aqueous solution of KOH (3 M) and 0.2 ml of methanol. The mixture was refluxed under nitrogen atmosphere for 1 h. It was then cooled to room temperature, acidified with aqueous HCl (3 M) to pH ~2, and extracted with dichloromethane (3×2 ml). The combined organic fractions were dried over anhydrous $MgSO_4$, filtered, and the solvent was completely removed under reduced pressure in a rotary evaporator.

Derivatization

Aliquots of hydrolyzed and nonhydrolyzed dichloromethane fractions (2.0 mg) were dissolved in pyridine (100 μl) in capped vials followed by the addition of 60 μl of bis(trimethylsilyl)-trifluoroacetamide containing 1% of chlorotrimethylsilane. The reaction mixture was heated at 70°C for 30 min. It was then cooled to room temperature before GC-MS analysis.

GC-MS analysis

GC-MS analyses were performed on a Shimadzu PQ5050A GC-MS equipped with an AOC-5000 autoinjector and a DB-1 J&W capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and using helium as carrier gas (35 cm s^{-1}). The chromatographic conditions were as follows: injector temperature 290°C; oven initial temperature 80°C hold for 5 min; temperature rate 4°C min^{-1}; final temperature 285°C hold for 40 min. The transfer-line temperature was 290°C and a split ratio of 1:10 was used. The mass detector was operated at electron-impact mode (70 eV) with a scan range of *m/z* 30 to 600.

For semiquantitative analysis, the GC-MS equipment was calibrated with pure reference compounds, representative of the major extractives components (namely, hexadecanoic acid, hexadecan-1-ol, 16-hydroxyhexadecanoic acid, 2-hydroxyoctanoic acid, tetracosane, β-sitosterol, and trans-ferulic acid), relative to hexanedioic acid and tetracosane used as internal standards as described in the literature.[19] The respective response factors needed to obtain correct quantification of the peak areas were calculated as an average of 16 GC-MS runs.

Compounds were identified as trimethylsilyl (TMS) derivatives by comparing their mass spectra with the GC-MS spectral library (Willey 333.000) with data from the literature, and, when necessary, by injection of reference compounds.

Results and discussion

The extraction with acetone was carried out in order to read the total amount of polar and lipophilic extractives. The wood lipophilic fraction can be easily isolated from the more complex acetone extract by its dissolution in a small amount of dichloromethane.[28,29]

The total amount of extractives (acetone fractions) from *Eucalyptus camaldulensis* dry wood [3.72%, standard deviation (SD) = 0.028] is much higher than the values found for

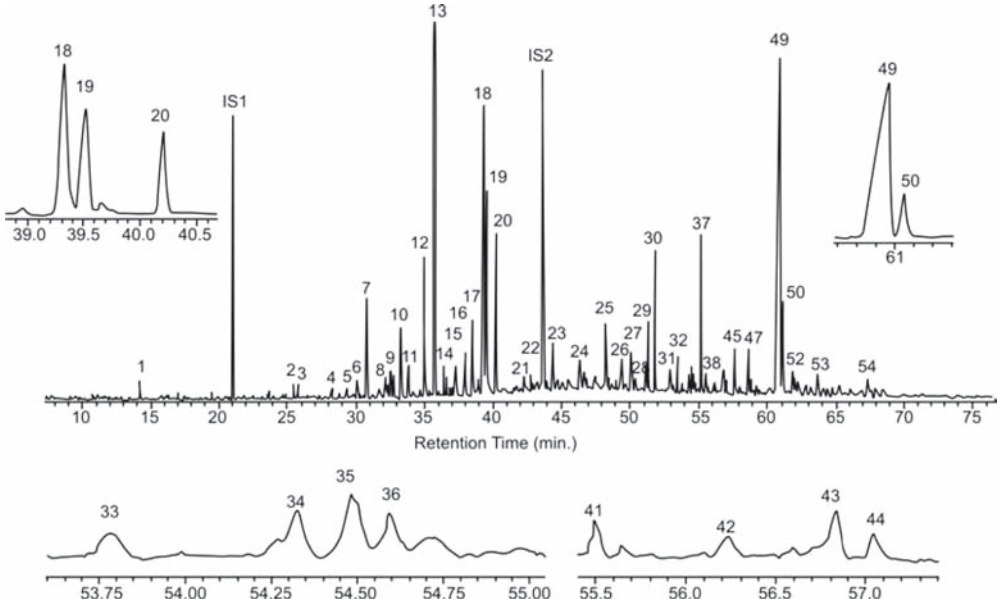

Fig. 1. Total ion chromatogram of the trimethylsilylated dichloromethane fraction of *Eucalyptus urograndis* wood. *IS1*, hexanedioic acid bis(trimethylsilyl) ester internal standard; *IS2*, tetracosane internal standard. Expansions of some parts of the chromatogram are included to show detail

Eucalyptus urograndis (1.32%, SD = 0.015) and *Eucalyptus urophylla* (2.93%, SD = 0.035); however, the dichloromethane-soluble fraction of the acetone fractions (lipophilic extractives) have shown quite similar yields, accounting for 0.47% (SD = 0.040) for *E. camaldulensis*, 0.38% (SD = 0.047) for *E. urograndis*, and 0.48% (SD = 0.041) for *E. urophylla*.

The amounts of lipophilic extractives found in the studied samples are significantly higher than the typical value (~0.26%) found for *Eucalyptus globulus*,[11,19] but at least in the case of *E. urograndis* it is in agreement with previously published results.[19]

The GC-MS analysis of derivatized dichloromethane fractions before and after alkaline hydrolysis of the three species revealed that they were quite similar from a qualitative point of view (Table 1). Figure 1 shows a typical total ion chromatogram obtained for the *E. urograndis* lipophilic fraction, after hydrolysis. A list of the identified compounds and their quantification, before and after hydrolysis, is shown in Table 1. The compounds identified in the hydrolyzed and nonhydrolyzed fractions could be grouped into three major classes according to their chemical structures, as shown in Fig. 2.

After alkaline hydrolysis, a large increase in the total amount of extractives detected by GC-MS was observed (Table 1, Fig. 2), particularly among the fatty acids and sterols. This confirms the presence of a significant amount of esterified structures such as steryl esters, glycerides, and waxes, among others, in the original extract,[8,9,11,13,19] in agreement with previous work.[4,6,7,11–14,19]

Fatty acids represented the major class of nonpolar components present in the lipophilic fraction of the extractives (after hydrolysis), with hexadecanoic acid (palmitic acid), octadeca-9,12-dienoic acid (linoleic acid), and octadec-9-enoic acid (oleic acid) as the major compounds, for all three species (Table 1), in agreement with previously reported

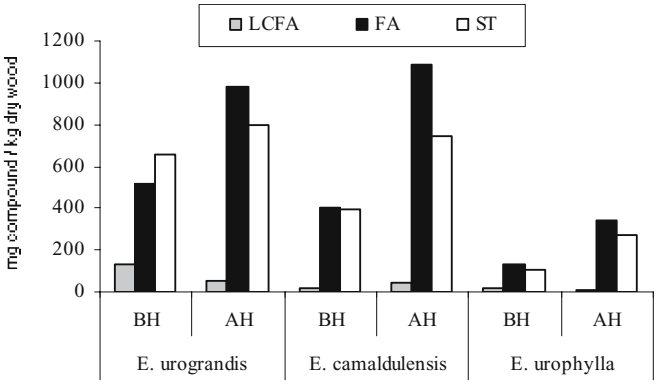

Fig. 2. Major classes of compounds present in the dichloromethane fractions of the investigated eucalyptus species, before (*BH*) and after alkaline hydrolysis (*AH*). *LCFA*, long-chain fatty alcohol; *FA*, fatty acids; *ST*, sterols

works for *E. globulus*.[11,13] Other identified fatty acids included nonanodioc, dodecanoic, tetradecanoic, pentadecanoic, heptadecanoic, octadecanoic, icosanoic, docosanoic, tricosanoic, tetracosanoic, pentacosanoic, hexacosanoic, heptacosanoic, and octacosanoic acids. Dodecanoic and tricosanoic acids were present in *E. urograndis* and *E. camaldulensis* (after hydrolysis), whereas heptacosanoic acid was only found in *E. urophylla* and *E. camaldulensis* after hydrolysis. Octacosanoic acid was also only found in *E. urograndis* (after hydrolysis) and *E. urophylla* (before hydrolysis). Additionally, five odd-number-chained fatty acids, namely pentadecanoic, heptadecanoic, tricosanoic, pentacosanoic, and heptacosanoic acids were identified in the wood extracts. Mass spectra of this class of compounds, once trimethylsilylated, present a characteristic fragmentation pattern showing major peaks at *m/z* 73 $[(CH_3)_3Si]^+$, 117 $[(CH_3)_3SiOC{=}O]^+$, 132 $[(CH_3)_3SiOC(=O)CH_3]^+$, and

Table 1. Lipophilic components identified in the dichloromethane fraction, before and after hydrolysis, of three species of *Eucalyptus* (*E. urograndis, E. camaldulensis*, and *E. urophylla*)

Peak[a]	Compound	[M+] (%)	Main fragments *m/z* (%)[c]	Eucalyptus urograndis		Eucalyptus camaldulensis		Eucalyptus urophylla	
				BH[b]	AH	BH	AH	BH	AH
1	Glycerol	308 (<1)	73(100), 147(32), 215(17)	97.3	4.63	9.40	8.40	3.80	7.30
2	Dodecanoic acid	272 (<1)	73(100), 117(47), 129(19), 257(18)		4.17		7.98		
3	4-Hydroxy-3,5-dimethoxybenzaldehyde	254 (100)	73(72), 224(100), 239(38), 254(18)	4.40	7.06				
4	4-Hydroxy-3-methoxybenzoic acid	312 (19)	78(100), 253(25), 267(31), 282(17), 297(43)	4.50	5.69				
5	Nonanodioic acid	332 (<1)	55(36), 73(100), 75(85), 117(17), 149(16), 201(10)	2.00	4.32		4.81		
6	Tetradec-9-enoic acid	298 (1.7)	73(100), 75(83), 117(43), 129(22), 283(4)		8.10				
7	Tetradecanoic acid	300 (<1)	73(100), 75(87), 117(60), 132(17), 129(24), 285(22)	5.50	33.7	16.9	41.3	2.40	10.9
8	cis-Ferulic acid	338 (17)	73(100), 293(10), 308(6), 323(10), 338(11)		12.6		13.9		
9	Pentadec-9-enoic acid	312 (<1)	73(88), 75(100), 117(52), 129(19), 132(6), 297(5)		2.97		28.1		
10	Pentadecanoic acid	314 (<1)	73(100), 75(86), 117(62), 129(24), 132(20), 299(20)	5.40	25.1	10.0	50.2	1.60	7.10
11	Hexadecan-1-ol	314 (<1)	43(52), 73(45), 75(100), 299(41)	4.40	6.47	2.60	9.87		3.50
12	Hexadec-9-enoic acid	326 (<1)	73(100), 75(93), 117(74), 129(28), 145(10), 311(22)	11.6	39.2	27.5	53.5	1.90	10.6
13	Hexadecanoic acid	328 (1.7)	73(100), 75(84), 117(65), 129(29), 145(14), 313(25)	125.4	161.4	115.9	189.2	46.5	77.6
14	trans-Ferulic acid	338 (29)	73(100), 293(16), 308(15), 323(20), 338(29)		20.7		24.8		
15	Heptadec-9-enoic acid	340 (<1)	73(96), 75(100), 117(41), 129(31), 145(10), 325(6)		10.3	6.40	13.4		
16	Heptadecanoic acid	342 (1.3)	73(100), 75(88), 117(69), 129(28), 145(14), 327(24)	5.00	11.6	3.90	9.05		4.00
17	Octadecan-1-ol	342 (<1)	43(56), 73(51), 75(100), 327(54)	13.9	39.1	5.90	23.6	2.70	
18	Octadeca-9,12-dienoic acid	352 (<1)	73(94), 75(100), 129(19), 337(11)	129.4	172.6	80.1	104.5	23.6	49.1
19	Octadec-9-enoic acid	354 (1.2)	73(96), 75(100), 117(49), 129(35), 145(13), 339(14)	68.5	145.8	92.1	159.9	21.3	64.3
20	Octadecanoic acid	356 (2.5)	73(100), 75(79), 117(63), 129(28), 145(15), 341(20)	27.9	71.1	25.2	82.7	16.9	47.9
21	NI hydrocarbon	–	43(84), 57(100), 71(51), 85(32), 99(8)	1.10	6.63			0.80	
22	Icosan-1-ol	270 (<1)	73(50), 75(100), 355(51)	9.30	3.41				
23	Icosanoic acid	384 (2.8)	73(100), 75(73), 117(63), 129(31), 145(18), 369(18)	5.00	6.75	6.60	24.3		7.30
24	NI hydrocarbon	–	43(89), 57(100), 71(69), 85(32), 99(10)	54.2	1.68			0.90	2.10
25	Docosanoic acid	412 (4.7)	73(100), 75(77), 117(70), 129(30), 145(20), 397(19)	15.9	14.4	4.40	24.1		9.70
26	NI hydrocarbon	–	43(88), 57(100), 71(71), 85(36), 99(10)	2.10	1.57	1.10			
27	Tricosanoic acid	426 (6.1)	73(100), 75(60), 117(71), 129(33), 145(22), 411(22)	15.0	12.1		8.15		
28	NI hydrocarbon	–	43(91), 57(100), 71(77), 85(44), 99(14)	2.40	2.10	1.00			2.50
29	Tetracosan-1-ol	426 (<1)	43(80), 73(63), 75(100), 411(70)		19.0				
30	Tetracosanoic acid	440 (5.1)	73(100), 75(76), 117(74), 129(32), 145(25), 425(22)	41.90	50.8	6.40	51.7	3.50	14.0
31	NI hydrocarbon	–	43(88), 57(100), 71(71), 85(43), 99(14)	2.10	1.98	1.50	11.4	4.0	2.70

32	Pentacosanoic acid	454 (4.9)	73(100), 75(67), 117(66), 129(29), 145(25), 439(16)	13.3	12.8		93.4		28.1
33	Hexacosan-1-ol	454 (<1)	43(81), 5763(), 73(61), 75(100), 439(60)	2.40	3.24				
34	2-Hydroxytetracosanoic acid	500 (<1)	73(100), 147(32), 411(23)		8.32		13.3		
35	22-Hydroxydocosanoic acid	500 (<1)	73(95), 75(100), 204(22), 217(12), 469(11), 485(16)		5.24		40.6		
36	NI hydrocarbon	–	43(89), 57(100), 71(72), 85(46), 99(14)	1.00	1.53				2.00
37	Hexacosanoic acid	468 (6.4)	73(100), 75(76), 117(70), 129(32), 145(28), 453(21)	38.6	78.5	2.20		7.60	
38	Stigmast-5-en-3-ol	414 (<1)	43(100), 55(67), 81(77), 145(46), 396(23)	6.00	34.2	48.7	25.1	8.00	
39	Heptacosanoic acid	482 (6.4)	73(100), 75(66), 117(68), 129(29), 145(22), 467(18)				34.2		7.40
40	NI steroid	–	43(93), 73(100), 165(88), 430(32)				13.3		28.9
41	NI steroid	–	43(93), 73(100), 165(88), 293(32), 341(33), 431(24)	32.6	7.65	2.10			
42	NI steroid	–	43(100), 73(94), 237(35), 503(24)	19.8	6.02	5.90			33.9
43	Cholestane-3,5-diol	548 (13)	73(91), 129(100), 329(43), 353(19), 368(34)		13.4	6.80	15.5		
44	NI steroid	–	55(100), 73(94), 129(69), 329(32), 368(22)		5.36	6.60			
45	24-Hydroxytetracosanoic acid	528 (<1)	73(95), 75(100), 204(22), 217(12), 497(10), 513(18)	5.7	15.2		16.4		
46	Octacosan-1-ol	482 (<1)	43(100), 57(86), 75(87), 467(50)					7.00	
47	Octacosanoic acid	496 (7.0)	73(100), 75(67), 117(66), 129(32), 145(31), 481(18)		29.8			4.60	
48	NI steroid	–	43(100), 73(80), 129(84), 343(29), 367(13), 382(29)					18.2	
49	*β*-Sitosterol	486 (9.9)	43(100), 73(83), 129(80), 357(24), 396(29)	512.0	608.3	251.2	504.5	67.5	196.5
50	*β*-Sitostanol	488 (8.6)	43(87), 75(100), 215(45), 473(11)	48.6	67.9	46.2	127.4	8.7	14.4
51	25-Hydroxypentacosanoic acid	542 (6.9)	73(88), 75(100), 204(24), 217(12), 527(13)				23.6		
52	26-Hydroxyhexacosanoic acid	556 (17)	73(95), 75(100), 204(22), 217(12) 541(8)		25.6				
53	Stigmasta-5,22-dien-3-ol	484 (22)	43(100), 135(58), 394(29)	22.7	28.6		62.9		
54	Stigmast-4-en-3-ona	412 (12)	43(94), 124(100), 229(29), 288(13)	14.8	29.0	22.5			
	Identified			1256.4	1853.2	790.9	1903.66	227.6	588.6
	Not identified			115.3	34.5	18.2	11.4	23.9	43.2
	Total			1371.7	1887.7	809.1	1915.1	251.5	631.8

When components remained unidentified, the compounds were partially characterized according to the chemical groups, based on their mass spectra
BH, Before hydrolysis; AH, after hydrolysis; NI, not identified
[a] Peak numbers refer to the chromatograms in Fig. 1
[b] Concentrations of lipophilic components given in units of (mg of compound/kg of dry wood)
[c] The fragments listed are those from the derivatized sample

$[M\text{-}CH_3]^+$.[13,30] An odd-number-chained dicarboxylic acid such as nonanodioic acid was also present in the lipophilic fractions of *E. urograndis* (before and after hydrolysis) and *E. camaldulensis* only after hydrolysis (Table 1).

Significantly higher amounts of fatty acids (approximately double) were found in the lipophilic fractions of *E. urograndis* and *E. camaldulensis* when compared with *E. globulus*[13] after hydrolysis, but at the same levels of *E. urophylla.* From the perspective of the pitch formation this looks to be an advantage of the latter species.

GC-MS analysis also allowed the identification of several ω-hydroxy fatty acids in small quantities; namely, 22-hydroxydocosanoic and 24-hydroxytetracosanoic acids in *E. urograndis* and *E. camaldulensis* wood extract (after hydrolysis), whereas 25-hydroxypentacosanoic acid has been identified only in *E. camaldulensis* and 26-hydroxyhexacosanoic acid only in *E. urograndis* after hydrolysis.

The ω-hydroxy fatty acids were identified as TMS derivatives, based on their characteristic fragmentation with mass spectra showing characteristic peaks of aliphatic TMS esters at *m/z* 117 $[(CH_3)_3SiOC{=}O]^+$, 129 $[(CH_3)_2Si{=}OC(=O)CH{=}CH_2]^+$, 204 $[C_8H_{20}O_2Si_2]^+$, and 217 $[C_9H_{21}O_2Si_2]^+$. The last two fragments, resulting from rearrangements of trimethylsilyl groups in long-chain aliphatic compounds, were consistent with the ω-hydroxy fatty acid or long-chain dicarboxylic acid structures.[31,32] However, the formation of fragments with *m/z* 89 and 103, characteristic of aliphatic alcohols, discard the possibility of being long-chain aliphatic dicarboxylic acids. In addition, the characteristic fragmentation pattern of the TMS derivatives of ω-hydroxy fatty acids was further confirmed by comparison with the mass spectrum of 16-hydroxyhexadecanoic acid (as TMS derivative) used as a reference sample.

Concerning α-hydroxy fatty acids, only 2-hydroxytetracosanoic acid has been identified in *E. urograndis* and *E. camaldulensis* after hydrolysis. The mass spectra of the TMS derivatives of those compounds also contain signals characteristics of aliphatic TMS esters at *m/z* 117, 129, and the corresponding $[M\text{-}15]^+$ fragment. The base peak due to the fragment $[M\text{-}117]^+$ indicates the α-cleavage,[30–33] which confirms the presence of a hydroxyl group in the α-position of the fatty acids. Finally, the fragmentation pattern of the TMS derivatives of α-hydroxy fatty acids was also confirmed by comparison with a silylated reference sample of α-hydroxyoctanoic acid. Although α-hydroxy fatty acids do not generally occur as compounds in *Eucalyptus* wood lipophilic fractions, their presence has been previously reported in *E. globulus*.[11,13] It has been demonstrated that these α- and ω-hydroxy fatty acids are commonly found as abundant components of pitch deposits in European pulp mills.[13,34] The significantly lower abundance of α- and ω-hydroxy fatty acids in the wood of the *Eucalyptus* species cultivated in Brazil seems to be clearly beneficial as far as pitch formation is concerned.

Mass spectra of the sterol TMS derivatives exhibited their molecular ions, confirming the compounds' molecular weights. The fragmentation patterns of this class of compounds are very characteristic. The main peaks observed correspond to the fragments $[(CH_3)_3Si]^+$ (at *m/z* 73), $[M\text{-}CH_3]^+$, $[M\text{-}90]^+$, $[M\text{-}129]^+$, and $[(CH_3)_3SiOCH{=}CHCH_2]^+$ (at *m/z* 129).[30,35–37]

The most abundant sterols found in all dichloromethane fractions were β-sitosterol and β-sitostanol. The content of these two sterols also increased upon hydrolysis. In the case of *E. camaldulensis*, increases of 100.8% and 175.8% in the amounts of β-sitosterol and β-sitostanol were observed, respectively. For *E. urophylla* and *E. urograndis*, the observed increases in the β-sitosterol content were 191% and 19%, respectively. These results show that in the extracts of all three species, the sterols are mainly present in the esterified form. Cholestane-3,5-diol was found in *E. urograndis*, but only in the hydrolyzed extract and in *E. camaldulensis* before and after hydrolysis, whereas stigmast-5-en-3-ol was found in three wood lipophilic fractions (before and after hydrolysis).

The amount of sterols found in *E. urophylla* is similar to those reported for *E. globulus*,[11] while the quantities found in *E. urograndis* and *E. camaldulensis* are significantly higher.

Long-chain fatty alcohols represented only a small portion of the total extractives identified by GC-MS. Octadecan-1-ol and hexadecan-1-ol were the main alcohols found in the analyzed fractions. These compounds were reported in previous studies as components of *E. globulus* wood extractives.[11,13] The mass spectrum of their TMS derivatives showed a prominent peak at *m/z* 75 and another intense peak corresponding to the $[M\text{-}CH_3]^+$ fragment, which are characteristic of these types of compounds.[30]

Ferulic acid was the main aromatic compound identified, in very small amounts, in the extracts after hydrolysis of *E. urograndis* [12.6 mg kg^{-1} (cis-isomer), 20.7 mg kg^{-1} (trans-isomer)] and *E. camaldulensis* [13.9 mg kg^{-1} (cis-isomer), 24.8 mg kg^{-1} (trans-isomer)]. However, this compound was not found in the lipophilic fractions of *E. urophylla* (Table 1). This compound was identified as a TMS derivative, by comparison with the equipment mass spectral library and with literature data.[38–40] This implies that ferulic acid should be present in *E. urograndis* and *E. camaldulensis* wood esterified with other components, such as lignin or polysaccharides, not detectable by GC-MS analysis, rather than with fatty alcohols and ω-hydroxy fatty acids. The aromatic composition of the studied species is much simpler than that reported for *E. globulus*.[13]

Table 1 reveals that the amount of components identified in the hydrolyzed extractives of *E urograndis* and *E. camaldulensis* corresponds approximately to 50% and 41% of the total lipophilic extractives, respectively. These results are in agreement with the percentage of compounds identified in the extractives of *E. globulus* (44.6% after hydrolysis) using the same GC-MS methodology.[13] However, in the case of *E. urophylla* few (13.2% in the case of the hydrolyzed sample) of the compounds present in the lipophilic extractives were identified. This last analysis was repeated twice more yielding the same outcome. A possible explanation for these results could be that the compounds present in the lipophilic fractions of this species are more prone to polym-

erization, therefore leading to a smaller fraction of volatile compounds under the GC-MS analysis conditions. The smaller amount of identified lipophilic extractives found in *E. urophylla* could be an advantage in terms of pitch formation during the industrial pulping process.

Further research will be required to evaluate the effects of the wood lipophilic fractions during pulping and bleaching stages.

Conclusions

This report describes the identification and quantification of a large number of lipophilic components present in the lipophilic fractions of *Eucalyptus urograndis*, *Eucalyptus urophylla*, and *Eucalyptus camaldulensis*. *Eucalyptus camaldulensis* and *E. urograndis* have higher contents of fatty acids and sterols, especially β-sitosterol, compared with *E. urophylla.*

The lower abundance of fatty acids and sterols in the lipophilic fractions from *E. urophylla* wood determines that pitch formation problems will be less severe for this species than for the other two.

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