JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8279–8285 8279
DOI:10.1021/jf101174x

Miscanthus x giganteus Bark Organosolv Fractionation: Fate of Lipophilic Components and Formation of Valuable Phenolic Byproducts

Juan José Villaverde,*,† Alberto De Vega,‡ Pablo Ligero,‡ Carmen S. R. Freire,† Carlos Pascoal Neto,† and Armando J. D. Silvestre†

†Centre for Research in Ceramics and Composite Materials (CICECO) and Department of Chemistry, University of Aveiro, Aveiro 3810-193, Portugal, and ‡Department of Physical Chemistry and Chemical Engineering, University of A Coruña, A Coruña 15071, Spain

The behavior of *Miscanthus x giganteus* bark lipophilic extractives during three acid organosolv pulping processes (Acetosolv, formic acid fractionation, and Milox) was investigated. It was demonstrated that nearly 90% of the lipophilic extractives were removed from pulps by either dissolution in the organosolv liquors (fatty acids and alcohols) or extensive degradation (sterols). The organosolv liquors were found to be rich in vanillin, syringaldehyde, and ferulic, vanillic, and *p*-coumaric acids. The Acetosolv fractionation process was found to be the most efficient in the removal of lipophilic components from pulps, and it was also the process that generated higher amounts of valuable monomeric phenolic compounds that could be exploited within the biorefinery context.

KEYWORDS: *Miscanthus x giganteus*; organosolv fractionation; lipophilic extractives; GC−MS analysis

INTRODUCTION

Biomass is expected to become the major renewable resource for the production of chemicals, materials, fuels, and energy (without affecting food and feed supplies), within a future sustainable society, following the so-called biorefinery concept (*1*−*3*).

To meet that vision, i.e., to use the available biomass as efficiently as possible and with the lowest environmental impact, deep scientific and technological improvements will be necessary covering all steps of the production chain, from agro-forest activities to the conversion of raw materials into valuable chemicals/materials (*1*−*3*). The selection of adequate plant species and of suitable processing technologies for their conversion are two crucial aspects to meet such goals.

The genus *Miscanthus* and, particularly, the sterile hybrid horticultural genotype *Miscanthus x giganteus* Greef and Deuter ex Hodkinson and Renvoize (*4*) have attracted the interest of the European Union (EU) authorities as a promising crop for energy and materials production, because of its excellent productivity, rapid growth, and high resistance to disease (*5*). In the past decade, numerous studies have been carried out to access the potential of this grass in a wide range of applications (*6*−*14*). Most of the mentioned applications require the fractionation of raw material into their main macromolecular components and were primarily based on pulping processes (*15*−*17*). Among them, organosolv fractionation processes have demonstrated to be promising alternatives to exploit some of these biomass resources for the production of methylcellulose (*13*), carboxymethylcellulose (*6*), adhesives (*7*), ethanol (*14*), and activated carbon (*9*), among other products (*8*).

*To whom correspondence should be addressed. Telephone: +351-234-370-711. Fax: +351-234-370-084. E-mail: jjvillaverde@ua.pt.

In this vein, we have studied the behavior of *M. x giganteus* bark during organosolv fractionation, with mixtures of acetic acid, water, and hydrochloric acid (Acetosolv process) (*16*, *17*), with formic acid, water, and hydrochloric acid as catalysts (henceforth referred as the Formosolv process) (*15*, *17*), and with mixtures of formic acid, water, and hydrogen peroxide (Milox process) (*18*). The totally chlorine-free (TCF) bleaching of the ensuing pulps (*19*, *20*) and the characterization of their main macromolecular components (*11*, *12*) were also investigated. Besides the macromolecular fractions, the exploitation of the extractives fraction could also contribute to the global valorization of the plant biomass. In this perspective, we have reported the first detailed characterization of the lipophilic extractives of *M. x giganteus* (*11*), showing that this species could be seen as a promising source of low-molecular-weight components, such as sterols and aromatic compounds.

To our knowledge, the behavior of *M. x giganteus* lipophilic components during the pulping and bleaching processes mentioned above has not been studied thus far; however, this knowledge is particularly important, on the one hand, in the perspective of their potential exploitation in an integrated biorefinery and, on the other hand, considering their potential impact in the pulping and bleaching processes.

It is known that the presence of lipophilic components causes problems in the pulp industry, such as the formation of deposits in machinery and dark spots in bleached pulp, known as pitch (e.g., refs *21*−*25*), as well as some increase in chemicals consumption during pulping and bleaching (*26*). Furthermore, extractives and their derivatives may impact bleaching effluents toxicity (e.g., refs *27* and *28*). However, these studies were in most cases focused on kraft pulping processes; therefore, a substantial lack of information exists when considering the behavior of these components during organosolv fractionation processes.

Published on Web 07/02/2010

Table 1. General Conditions of *M. x giganteus* Bark Organosolv Fractionation and General Properties of Corresponding Organosolv Pulps

		fractionation process		
		Acetosolv	Formosolv	Milox
experimental conditions	pulping liquor (w/w/w)	$CH_3CO_2H/H_2O/HCl$ (90:9.85:0.15)	$HCO_2H/H_2O/HCl$ (90:9.90:0.10)	$HCO_2H/H_2O/H_2O_2$ (85:14.5:0.5)
	liquid/bark ratio (w/w)	12	12	20
	temperature/time	boiling/55 min	boiling/36 min	first stage: 63 °C/45 min second stage: boiling/60 min
pulp properties	pulp yield (%)	51.9	48.7	55.4
	κ number	18.1	23.0	19.1
	viscosity (cm^3/g)	998	1197	902
	brightness (% ISO)	33.4	30.6	41.1

In view of the renewed interest of fractionation processes related to the development of the biorefinery concept and the predictable differences in the behavior of lipophilic compounds under organosolv conditions, the analysis of the lipophilic fraction behavior is of major importance. A study of this type can provide new insights into the extent of their removal and degradation during fractionation and contribute to the integrated exploitation of some of them and the solution of pitch formation problems.

The objective of this work was to study the behavior of the lipophilic components of *M. x giganteus* bark, following our interest in the organosolv fractionation by Acetosolv, Formosolv, and Milox processes and covering the analysis of these fractions in the resulting pulps and pulping liquors.

MATERIALS AND METHODS

Chemicals. Potassium hydroxide (puriss, p.a., ≥86%) and methanol (puriss, p.a., absolute, ≥99.8% purity) were purchased from Fluka Chemie (Madrid, Spain). Dichloromethane (DCM, 99% purity), pyridine (99% purity), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (99% purity), trimethylchlorosilane (99% purity), stigmasterol (95% purity), octadecanoic acid (99% purity), nonadecanol (99% purity), coniferyl alcohol (98% purity), and tetracosane (99% purity) were supplied by Sigma Chemicals Co. (Madrid, Spain). Finally, formic acid (98.0% purity), glacial acetic acid (99.5% purity), hydrochloric acid (puriss, p.a., fuming, ≥37%), and hydrogen peroxide (30% p/v) were provisioned by Panreac (Barcelona, Spain).

Raw Material. *M. x giganteus* plants were randomly sampled from an experimental plantation in Santiago de Compostela (Spain), established as part of the EU AIR *Miscanthus* Productivity Network (*5*). A detailed description of the characteristics of this material has been reported elsewhere (*4*). The plants were manually stripped of leaves and core, air-dried for 2 weeks, and ground to pass through a 1 cm sieve. The ground bark was allowed to attain its equilibrium humidity (7.7%) and finally stored in hermetic polypropylene containers.

Organosolv Fractionation. Acetosolv, Formosolv, and Milox fractionations of *M. x giganteus* bark were carried out as previously described (*15*−*18*) using the experimental conditions reported in **Table 1**. Ground *M. x giganteus* bark was refluxed with the pulping liquor (in the case of the Milox system, only the second stage reached the boiling temperature). Then, the pulp was filtered and washed 4 times with 85% acetic acid or 80% formic acid, depending upon the fractionation process (in w/v proportions of 0.4, 0.4, 0.2, and 0.2 with respect to the initial dry weight of *M. x giganteus*). The black liquors were separated, and pulps were washed repeatedly with distilled water until neutrality was achieved. These experimental conditions were setup to obtain pulps with κ numbers around 20.

Characterization of Organosolv Pulps. For all pulps, κ numbers (KN) and ISO brightness (BR) were measured according to Tappi standards (T236 and T525, respectively). Intrinsic viscosities (VIS) were determined according to UNE-039-92, and pulp yields (PY) were determined gravimetrically after oven drying until they reached a constant weight. A detailed discussion of these results is reported elsewhere (*17*, *18*).

Solvent Extraction. Three aliquots (20 g) of each dried organosolv pulp were extracted in a Soxhlet extractor with DCM (200 cm^3) for 8 h. DCM was chosen because it is a fairly specific solvent for lipophilic extractives (*11*). The solvent was evaporated to dryness, and the extracts were weighed.

Pulping liquors, three aliquots (5 cm^3) of each one, were first submitted to lignin precipitation by water addition (water/liquor = 5:1, v/v) (*12*, *18*). The mixture was then centrifuged, and the lignin was separated from the liquid phase. The lipophilic extractives present in the supernatant were isolated by liquid−liquid extraction with DCM (3 × 15 cm^3). The precipitated lignin was also washed with DCM to remove co-precipitated extractives. The two DCM extracts were combined and dried over anhydrous sodium sulfate; the solvent was evaporated and the extract was quantified gravimetrically. The results were expressed as a percentage of dry material.

Alkaline Hydrolysis. About 20 mg of each extract was dissolved in 10 cm^3 of 1 M KOH in 10% aqueous methanol. The mixtures were heated at 100 °C, under nitrogen, for 1 h. The reaction mixtures were cooled, acidified with 1 M HCl to pH 2, and then extracted 3 times with DCM. The solvent was evaporated to dryness.

Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. Before GC−MS analysis, approximately 20 mg of each dried sample and 0.25−0.50 mg of internal standard (tetracosane) were dissolved in 200 μL of pyridine and the compounds containing hydroxyl and carboxyl groups were converted into trimethylsilyl (TMS) ethers and esters, respectively, by adding 250 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide and 50 μL of trimethylchlorosilane. After the mixture had remained at 70 °C for 30 min, the derivatized extracts were analyzed by GC−MS following previously described methodologies (*11*, *29*, *30*). The GC−MS analysis was performed on a trace gas chromatograph 2000 series, equipped with a Thermo Scientific DSQII single-quadrupole mass spectrometer. Analysis of samples, before and after alkaline hydrolysis, were carried out using a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness). The chromatographic conditions were as follows: initial temperature, 80 °C for 5 min; temperature gradient, 4 °C/min; final temperature, 260 °C; temperature gradient, 2 °C/min; final temperature, 285 °C for 8 min; injector temperature, 250 °C; transfer-line temperature, 290 °C; split ratio, 1:33 (*11*, *29*, *30*).

To check the presence of esterified structures, samples were also analyzed in a DB-1 J&W capillary column (15 m × 0.32 mm inner diameter, 0.25 μm film thickness); the chromatographic conditions were as follows: initial temperature, 100 °C for 3 min; temperature gradient, 5 °C/min; final temperature, 340 °C for 12 min; injector temperature, 290 °C; transfer-line temperature, 290 °C; split ratio, 1:33 (*11*, *29*, *30*).

Chromatographic peaks were identified on the basis of the comparison of their mass spectra with the equipment mass spectral library (Wiley-NIST Mass Spectral Library 1999), their characteristic retention times obtained under the described experimental conditions (*11*, *29*, *30*), and their fragmentation profiles with published data (refs *11*, *29*, and *30* and references therein).

For quantitative analysis, GC−MS was calibrated with pure reference compounds, representative of the major lipophilic extractive components (namely, coniferyl alcohol, octadecanoic acid, nonadecanol, and stigmasterol), relative to tetracosane. The respective response factors were calculated as an average of six GC−MS runs. Two aliquots of each extract were analyzed before alkaline hydrolysis and another two after alkaline hydrolysis. Each aliquot was injected in triplicate. The presented results are the average of the concordant values obtained for each sample (less than 5% variation between injections of the same aliquot and between aliquots of the same sample).

RESULTS AND DISCUSSION

The general characteristics of the obtained organosolv pulps used in this study (**Table 1**) were discussed in detail elsewhere (*15*−*18*), and the DCM extraction yields are shown in **Table 2**, being the

Table 2. Extraction Yields for *M. x giganteus* Bark (Percentage of Extracts, on a Dry Bark Basis) and Its Acid Organosolv Pulps (Percentage of Extract Per Mass of Dry Pulp) and Pulping Liquors (Percentage of Extract Per Mass of Dry Bark)[a]

	lipophilic extractives (%)	
	bark/pulp	pulping liquors
M. x giganteus bark	0.53	
Acetosolv pulp	0.14	17.3
Formosolv pulp	0.15	7.7
Milox pulp	0.11	10.9

[a] The results are the average of three replicates with less than 5% variability.

average of three replicates with less than 5% variability. These pulps were obtained in similar yields to those obtained by kraft pulping of wood (e.g., eucalyptus and pine), with their intrinsic viscosities above 900 cm^3/g, κ numbers around 20, and their brightness (mainly Milox pulp) high when compared to typical bleachable kraft pulps (around 25% ISO) (*31*, *32*). Therefore, they have very suitable properties to start a bleaching process.

The reported DCM extraction yields (**Table 2**) found for *M. x giganteus* unbleached organosolv pulps show that the organosolv fractionation processes promoted a substantial decrease in the amounts of DCM extractives, from around 0.5% in the raw bark (*11*) to 0.11−0.15% in the studied organosolv pulps. If reported to the initial mass of bark and considering the described pulping yields (**Table 1**), this means that only 11.5−13.8% of the DCM extractives present in bark were retained in the pulps. The rest of these components (nearly 90%) were predominantly removed with the pulping liquors. The removal percentages were considerably higher than those reported for kraft pulping of *Eucalyptus globulus*, with only around 53% of the lipophilic extractives removed (*23*), or for soda/AQ pulping of *Cannabis sativa*, *Agave sisalana*, and *Musa textilis*, with 70, 56, and 61% of the lipophilic extractives removed, respectively (*33*). To our knowledge, only soda/AQ pulping of *Linum usitatissimum*, with a removal of 89% (*33*), presented similar removing efficiencies for lipophilic components.

The higher efficiency in the removal of lipophilic components observed and, particularly, when compared to the kraft process (*23*) is likely related to the higher solubility of this fraction in the organic media involved in the organosolv fractionation. The low contents of lipophilic components will obviously have a beneficial impact in the bleaching stages because of the potential reduction of the pitch formation and the consumption of bleaching chemicals as referred above.

Considering the above-mentioned results and the possible interest on the exploitation of some of these components, it is therefore important to study in detail the composition of the pulping liquors. The DCM extraction yields of the pulping liquors (**Table 2**) accounted for 7.7, 10.9, and 17.3% in relation to the starting bark dry mass, for Formosolv, Milox, and Acetosolv, respectively. Obviously, these high yields are mainly due to the presence of oligomeric lignin degradation products (*34*) and not directly due to bark lipophilic components, as will be discussed bellow.

Lipophilic Extractives in Organosolv Pulps. The results of the GC−MS analysis of the lipophilic extracts of Acetosolv, Formosolv, and Milox pulps are shown in **Table 3** and **Figure 1**. Only the results of the analysis after alkaline hydrolysis are shown; however, it was observed that, in general, only a slight increment (3−5%) with respect to non-hydrolyzed material in the amount of detected compounds was observed, in line with the small amounts of esterified structures previously detected in the raw bark (*11*) and also with the possibility of partial acid hydrolysis during the organosolv fractionation step. From a qualitative point of view, these extracts are quite similar to those of the untreated bark (*11*). Fatty acids were the predominant family found (with hexadecanoic, octadecanoic, and octacosanoic acids as the major components), followed by aromatic compounds (with vanillin, syringaldehyde, *p*-hydroxybenzaldehyde, and ferulic and *p*-coumaric acids as the major components), smaller amounts of fatty alcohols (mainly hexadecan-1-ol, octadecan-1-ol, and octacosan-1-ol), and trace amounts of sterols (predominantly, β-sitosterol). Sterols, because of their higher lipophilic character, were expected to be preferentially retained in pulps; however, this was not confirmed with the present results, which means that they were either solubilized or degraded during organosolv fractionation, as will be discussed below.

Finally, the analysis of the lipophilic extractives of pulps by GC−MS using short length columns confirmed the low amounts of esterified structures (steryl glucosides and steryl esters) suggested above and also that 1,3-diferuloylglycerol, reported as a bark component (*11*), was only detected in trace amounts in Acetosolv pulps.

Quantitatively, all organosolv pulps showed much lower amounts of lipophilic compounds (**Figure 1**) than those found in bark. Acetosolv pulps showed the lowest amounts of these compounds, followed by Milox and Formosolv pulps. The main variations in the amounts of lipophilic components in the different pulps were assigned to fatty acids, which appeared in notoriously higher amounts in the Formosolv pulps (**Figure 1**), followed by the aromatic fraction (which is also influenced by lignin degradation) and long-chain fatty alcohols. Sterols appeared at low levels in all of the studied pulp extracts.

It is known that the behavior of lipophilic components during pulping processes is driven by their distribution between pulping liquors and pulps and their degradation during this stage (*23*,*35*). In organosolv processes, the organic nature of the reaction media will obviously increase the removal of lipophilic components by solubilization. Additionally, in the case of Milox, there are also strongly oxidizing conditions (*18*) that could also induce degradation of lipophilic components (*23*). The contribution of oxidative processes to the removal of lipophilic components is clearly evidenced in the case of sterols, by the presence of trace amounts of oxidized derivatives (**Table 2**).

Thus, broadly, organosolv fractionation processes allow for a higher removal of lipophilic components than conventional kraft processes, reducing the risk of pitch formation during bleaching stages, and would be more beneficial for the fractionation of specific biomass sources with high extractive contents. Despite this general assumption, Formosolv pulps showed higher risks of generating pitch problems than Milox or Acetosolv because of its higher content of lipophilic components (**Figure 1**).

Composition in Lipophilic Extractives of Pulping Liquors. Considering the extensive removal of lipophilic components from *M. x giganteus* bark during organosolv fractionation and to better understand their behavior, the composition of the DCM fraction of organosolv liquors was also studied. The chemical compositions of the DCM extracts, after alkaline hydrolysis, of organosolv liquors are shown in **Table 3**. Acetosolv liquors showed the highest content on detected lipophilic components (18.0 g/kg of dry bark), followed by Milox and Formosolv liquors (9.6 and 6.5 g/kg of dry bark, respectively). These values were considerably higher than those reported for pulps because of the presence of considerable amounts of phenolic compounds arising from lignin degradation. However, the amounts of detected components were considerably lower than the total DCM extraction yields reported above, which should have been due to the fact that a substantial part of these extracts was composed of oligomeric structures resulting from lignin degradation, which are not detected under the chromatographic conditions used (*34*).

As expected, the organosolv liquor extracts have shown considerably high amounts of fatty acids and alcohols, thus confirming their important solubilization during organosolv fractionation with little deposition on pulp fibers.

Table 3. Compounds Identified in the DCM Extracts of *M. x giganteus* Organosolv Pulps, Expressed in Milligrams of Compound Per Kilogram of Dry Pulp and Black Liquors after Alkaline Hydrolysis, Expressed in Milligrams of Compound Per Kilogram of Dry Bark[a]

		pulps			black liquors		
tr (min)	compound	Acetosolv	Formosolv	Milox	Acetosolv	Formosolv	Milox
	fatty acids	57.5	156.3	83.4	594.6	448.0	501.8
	saturated	49.3	105.1	73.7	558.6	429.8	501.8
6.3	hexanoic acid	0.4	0.2	0.5	27.8	38.6	88.6
9.4	heptanoic acid	TR	TR	TR	2.0	ND	ND
12.7	octanoic acid	0.8	0.4	1.0	7.5	TR	TR
15.9	nonanoic acid	1.2	0.3	1.7	30.9	26.0	57.0
19.0	decanoic acid	0.7	0.2	0.7	TR	TR	ND
24.7	dodecanoic acid	1.5	0.3	3.0	13.7	12.7	29.7
27.3	tridecanoic acid	0.3	0.1	0.4	4.5	TR	TR
29.8	tetradecanoic acid	1.8	1.1	3.7	28.8	19.8	41.0
32.2	pentadecanoic acid	1.2	1.1	2.4	TR	TR	ND
34.5	hexadecanoic acid	14.6	45.4	16.9	287.9	185.2	178.8
36.7	heptadecanoic acid	2.1	0.6	0.6	5.6	10.6	8.5
38.8	octadecanoic acid	5.9	6.2	3.5	104.5	64.7	55.6
40.9	nonadecanoic acid	0.1	0.2	0.2	ND	0.5	7.4
42.8	eicosanoic acid	0.9	3.7	2.8	14.9	TR	TR
44.7	heneicosanoic acid	0.3	0.5	0.4	4.0	4.6	9.5
46.5	docosanoic acid	1.6	3.2	1.8	6.4	9.9	9.5
48.3	tricosanoic acid	0.8	2.7	1.9	5.2	13.1	6.7
50.0	tetracosanoic acid	1.5	5.3	3.6	14.9	44.1	9.5
51.7	pentacosanoic acid	0.6	1.4	1.0	ND	ND	ND
53.6	hexacosanoic acid	1.5	3.0	2.3	TR	ND	ND
55.6	heptacosanoic acid	0.9	2.2	1.2	ND	ND	ND
57.6	octacosanoic acid	9.3	23.3	20.9	ND	ND	ND
59.8	nonacosanoic acid	TR	TR	TR	ND	ND	ND
62.0	triacontanoic acid	1.6	3.7	3.2	ND	ND	ND
	unsaturated	7.6	40.1	7.3	36.0	18.2	TR
33.8	*trans*-9-hexadecenoic acid	1.4	0.6	2.9	ND	ND	ND
38.0	9,12-octadecadienoic acid	1.5	9.4	0.8	ND	ND	ND
38.2	*cis*-9-octadecenoic acid	4.0	29.1	3.2	20.5	18.2	ND
38.3	*trans*-9-octadecenoic acid	0.7	1.0	0.4	15.5	TR	TR
	α-hydroxy acids	0.6	11.1	2.4	TR	TR	TR
46.0	2-hydroxyeicosanoic acid	ND	ND	ND	TR	TR	ND
49.4	2-hydroxydocosanoic acid	0.2	2.6	0.4	TR	TR	ND
51.1	2-hydroxytricosanoic acid	TR	TR	TR	TR	TR	TR
52.9	2-hydroxytetracosanoic acid	0.4	8.5	2.0	ND	ND	ND
	aromatic compounds	36.8	8.2	49.1	16579.2	5825.8	8826.5
11.6	benzoic acid	1.6	0.3	3.2	44.3	66.1	121.2
15.5	*p*-hydroxybenzaldehyde	5.6	1.2	35.3	283.5	189.4	733.3
17.3	resorcinol	TR	TR	TR	TR	5.8	ND
18.5	acetophenone	ND	ND	ND	54.7	39.8	44.4
20.3	vanillin	7.1	1.5	4.9	5742.9	2231.1	4463.4
21.5	4-hydroxypropiophenone	ND	ND	ND	13.0	9.2	22.0
23.8	4-hydroxybenzoic acid	TR	TR	TR	4.1	ND	ND
25.0	syringaldehyde	7.8	0.8	2.1	2406.2	2117.4	1387.2
27.4	vanillic acid	2.2	0.7	1.2	1094.2	287.5	713.2
27.5	3-methoxy-4-hydroxyphenylacetic acid	ND	ND	ND	56.8	53.6	108.0
28.6	4-hydroxy-3-methoxycinnamaldehyde	ND	ND	ND	14.9	ND	13.6
28.9	3-vanillylpropanol	ND	ND	ND	108.6	31.2	TR
29.0	3-syringyl-3-oxopropanal	ND	ND	ND	99.5	33.9	38.2
30.1	3-hydroxy-1-(vanillyl)propan-1-one	ND	ND	ND	1336.7	7.0	ND
30.7	syringic acid	2.0	0.4	0.6	386.9	204.5	285.0
31.1	1-guaiacyl-2-hydroxyethanone	ND	ND	0.2	28.2	8.0	6.5
31.7	*p*-coumaric acid	5.0	0.8	0.7	1453.8	27.1	69.8
32.5	3,5-dimethoxy-4-hidroxycinnamaldehyde	ND	ND	ND	TR	TR	ND
33.3	guaiacylglyoxylic acid	ND	ND	TR	14.5	TR	TR
34.1	2-hydroxy-1-syringylethanone	ND	ND	TR	ND	ND	ND
35.2	ferulic acid	5.5	2.5	0.9	3436.4	514.2	820.7
	sterols	9.4	5.2	3.1	TR	TR	TR
55.3	cholesterol	1.5	2.3	2.0	ND	ND	ND
58.1	stigmasterol	1.9	0.8	1.1	ND	ND	ND
59.4	β-sitosterol	6.0	2.1	TR	TR	TR	TR
59.6	stigmasta-3,5-dien-7-one	TR	TR	TR	ND	ND	ND
61.8	7-hydroxy-β-sitosterol	TR	ND	ND	TR	TR	TR
65.7	7-oxo-β-sitosterol	TR	TR	TR	ND	ND	ND

Table 3. Continued

tr (min)	compound	pulps			black liquors		
		Acetosolv	Formosolv	Milox	Acetosolv	Formosolv	Milox
	fatty alcohols	42.4	25.5	25.7	28.4	58.2	57.2
	saturated	26.8	25.5	25.7	28.4	58.2	57.2
32.8	hexadecan-1-ol	5.8	0.1	0.5	10.6	58.2	57.2
37.2	octadecan-1-ol	4.2	0.1	0.6	ND	ND	ND
39.3	nonadecan-1-ol	0.5	0.1	0.5	ND	ND	ND
52.1	hexacosan-1-ol	0.8	2.7	1.6	17.8	TR	ND
53.9	heptacosan-1-ol	0.4	1.2	0.8	ND	ND	ND
55.9	octacosan-1-ol	15.1	21.3	21.7	TR	TR	TR
	unsaturated	15.6	ND	ND	ND	ND	ND
36.5	9-octadecen-1-ol	15.6	ND	ND	ND	ND	ND
	others	2.3	6.0	3.8	792.8	90.2	193.0
5.7	2-furanecarboxylic acid	ND	ND	ND	187.2	TR	TR
6.2	2-hydroxypropanoic acid	0.2	0.1	TR	27.8	27.9	62.7
7.6	levulinic acid	TR	TR	TR	5.5	3.0	49.4
8.8	3-hydroxypropanoic acid	TR	TR	TR	ND	ND	ND
12.2	4-methylpentan-1,2-diol	ND	ND	0.3	ND	ND	ND
12.8	pentan-1,5-diol	ND	ND	TR	ND	ND	ND
14.0	glycerol	TR	0.5	TR	7.3	8.5	19.5
25.5	pentadecan-2-one	ND	ND	ND	545.5	28.9	42.1
25.8	octanedioic acid	ND	TR	TR	5.5	8.6	TR
28.4	azelaic acid	0.3	0.3	0.3	14.0	13.3	19.3
47.7	heptacosane	0.8	2.6	2.1	ND	ND	ND
53.3	octadecanal	0.8	0.7	0.6	ND	ND	ND
54.7	pentacosan-1,2-diol	0.2	1.8	0.5	ND	ND	ND
	non-identified compounds	0.5	0.2	1.1	13.9	31.1	43.6

[a] Results are the average of the concordant values obtained (less than 5% variation between injections) for the two aliquots of each sample injected in triplicate (ND, not detected; TR, trace).

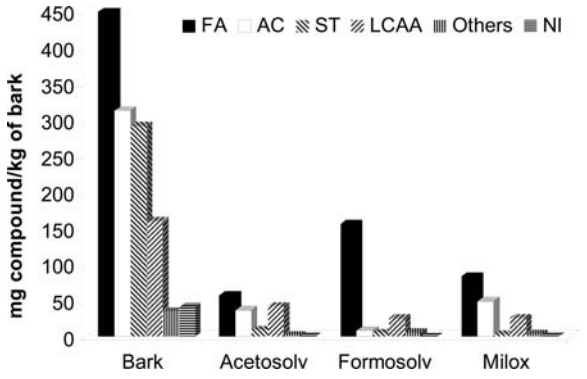

Figure 1. Major families of identified components in DCM extracts of *M. x giganteus* organosolv bark and pulps, after alkaline hydrolysis. FA, fatty acids; AC, aromatic compounds; ST, sterols; LCAA, long-chain aliphatic alcohols; NI, not identified.

Surprisingly sterols were nearly vanished from pulping liquors. This observation, together with their low content in pulps and the fact that only trace amounts of partially oxidized sterol derivatives (*23*) were detected, points out that this family should have been extensively oxidized during the organosolv stages, excluding the possibility of using organosolv liquors as a stream to recover *M. x giganteus* bark sterols as previously suggested (*11*). In this perspective, the exploitation of bark sterols would imply their extraction before organosolv fractionation; furthermore, this could be performed together with the core, where these compounds are abundantly found (*11*).

Among all results showed in **Table 3**, the aromatic compound content was remarkably high, especially in the Acetosolv liquors. This converts them into important sources for this family of compounds, especially for vanillin in the three organosolv processes, with 5.7, 2.2, and 4.5 g/kg of dry bark, for Acetosolv, Formosolv, and Milox systems, respectively, followed by syringaldehyde (2.4, 2.1, and 1.4 g/kg of dry bark). Additionally, *p*-coumaric acid, ferulic acid, and 3-hydroxy-1-(vanillyl)propan-1-one (1.5, 3.4, and 1.3 g/kg of dry bark, respectively) were also abundant components of the Acetosolv liquor, being minor components in the others. These phenolic compounds were either absent or found in considerably smaller amounts in the starting bark (*11*), which means that they were mainly resulting from lignin degradation reactions. After alkaline hydrolysis, a slight increase (~4%) in the amount of some phenolic compounds was measured. This result is consistent with the presence of olygomeric lignin-derived structures that released their phenolic part upon saponification.

In addition to the phenolic compounds previously detected in *M. x giganteus* bark, two other phenolic components were detected at tr = 29.0 and 30.1 min (the last one being a major component of Acetosolv liquor). These two peaks were tentatively identified as follows. The peak at 29.0 min corresponds to a syringyl derivative, with a carbonyl group adjacent to the aromatic moiety (as a TMS derivative), confirmed by the presence in its mass spectra (**Figure 2a**) of a characteristic fragment at *m*/*z* 253 (*35*). This information, together with the molecular ion at *m*/*z* 296 and the corresponding [M − CH_3] fragment at *m*/*z* 281, is compatible to a 3-syringil-3-oxopropanal structure.

The peak at 30.1 min corresponds to a vanillyl derivative, with a carbonyl group adjacent to the aromatic moiety (as a TMS derivative), confirmed by the presence in its mass spectra (**Figure 2b**) of a characteristic fragment at *m*/*z* 223 (*35*). Furthermore, the fragment at *m*/*z* 117 is compatible to a [CH_2CH_2OTMS] fragment. Both results in conjunction with a molecular ion at *m*/*z* 340 and a M − CH_3 ion at *m*/*z* 325 are compatible to 3-hydroxy-1-(vanillyl)propan-1-one as a di-TMS derivative.

Behavior of Extractives during Organosolv Fractionation. A general overview of the behavior of the studied components during organosolv fractionation can be obtained from **Table 4**.

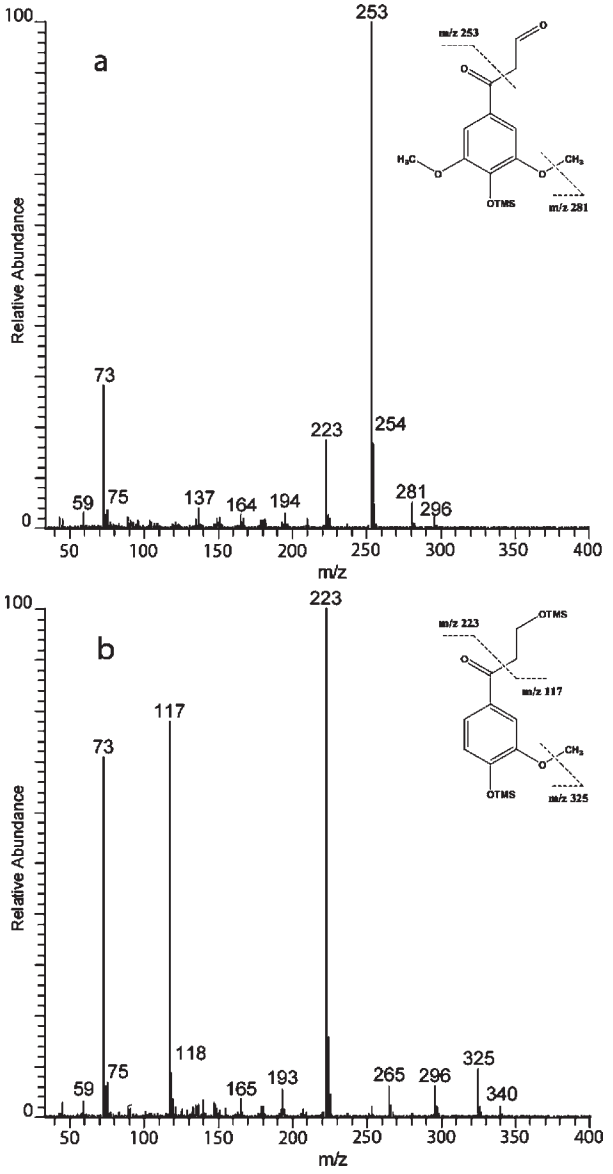

Figure 2. Mass spectra of the TMS derivatives of (a) 3-syringil-3-oxopropanal and (b) 3-hydroxy-1-(vanillyl)propan-1-one.

As mentioned above, the total amount of phenolic compounds found in pulps and liquors is considerably higher than in the starting bark, because most of them are formed during lignin degradation. In the case of sterols, the total amounts detected in pulps and liquors were considerably lower than those found in bark, which suggests that these components are extensively degraded during organosolv fractionation.

Finally, fatty acids and alcohols were mainly found in liquors, but the total amounts of fatty acids detected in pulps and liquors were slightly higher than those found in the starting bark, whereas in the case of fatty alcohols, the opposite was observed. This suggests that oxidation of fatty alcohols into fatty acids may have contributed to the decrease of the former and the increase of the latter.

It can be concluded from this study that *M. x giganteus* bark lipophilic components were efficiently removed from pulps during the organosolv fractionation, by either dissolution (for fatty acids, long-chain aliphatic alcohols, and aromatic compounds) or degradation in the case of sterols. The results showed that organosolv processes could be efficiently used for the fractionation of lignocellulosic materials when the removal of lipophilic extractives is required to reduce potential problems of pitch deposition in later stages of pulp processing (e.g., during bleaching).

Table 4. Major Families of Components Identified in the DCM Extracts of *M. x giganteus* Bark and Their Distribution in the Pulps and Black Liquors after Acetosolv, Formosolv, and Milox Fractionation after Alkaline Hydrolysis (Expressed in Milligrams of Compound Per Kilogram of Dry Bark)[a]

		FA	AC	ST	LCAA
bark		450	313	292	160
pulps	Acetosolv	30	19	5	22
	Formosolv	76	4	3	12
	Milox	46	27	2	14
liquors	Acetosolv	595	16579	TR	28
	Formosolv	448	5826	TR	58
	Milox	502	8827	TR	57

[a] FA, fatty acids; AC, aromatic compounds; ST, sterols; LCAA, long-chain aliphatic alcohols.

Additionally, pulping liquor extracts were found to be quite rich in valuable monomeric phenolic compounds, such as syringaldehyde, vanillic acid, vanillin, and *p*-coumaric acid, and could therefore be seen as a good source of these components toward the valorization of organosolv liquors in a biorefinery perspective.

On the contrary, sterols are nearly absent in organosolv liquors, and therefore, their exploitation, in the perspective of the global valorization of *Miscanthus* biomass, will only be possible through extraction prior to organosolv fractionation.

In general, Acetosolv was the most efficient process in terms of removal of lipophilic components from pulps, as well as in the generation of low-molecular-weight phenolic components.

Future work will be carried out to study the oligomeric phenolic fraction of the DCM extracts to increase the knowledge on the composition of these organosolv liquors and contribute to their valorization.

ACKNOWLEDGMENT

The authors thank Dr. D. Manuel Bao Iglesias, from the University of Santiago de Compostela, for kindly supplying *M. x giganteus*.

LITERATURE CITED

(1) Fernando, S.; Adhikari, S.; Chandrapal, C; Murali, N. Biorefineries: Current status, challenges, and future direction. *Energy Fuels* **2006**, *20*, 1727–1737.

(2) Kamm, B.; Kamm, M. Principles of biorefineries. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 137–145.

(3) Kamm, B.; Kamm, M.; Gruber, P. R.; Kromus, S. Biorefinery systems - An overview. In *Biorefineries - Industrial Processes and Products*; Kamm, B., Gruber, P. R., Kamm, M., Eds.; Wiley-VCH: Weinheim, Germany, 2006; pp 3−40.

(4) Hodkinson, T. R.; Renvoize, S. Nomenclature of *Miscanthus x giganteus* (Poaceae). *Kew Bull.* **2001**, *56*, 759–760.

(5) Lewandowski, I.; Clifton-Brown, J. C.; Scurlock, J. M. O.; Huisman, W. *Miscanthus*: European experience with a novel energy crop. *Biomass Bioenergy* **2000**, *19*, 209–227.

(6) Barba, C.; Montane, D.; Rinaudo, M.; Farriol, X. Synthesis and characterization of carboxymethylcelluloses (CMC) from non-wood fibers I. Accessibility of cellulose fibers and CMC synthesis. *Cellulose* **2002**, *9*, 319–326.

(7) El Mansouri, N.-E.; Salvadó, J. Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and etanol process lignins. *Ind. Crops Prod.* **2006**, *24*, 8–16.

(8) Lundquist, L.; Arpin, G.; Leterrier, Y.; Berthold, F.; Lindström, M.; Månson, J.-A. E. Alkali-methanol-anthraquinone pulping of *Miscanthus x giganteus* for thermoplastic composite reinforcement. *J. Appl. Polym. Sci.* **2004**, *92*, 2132–2143.

(9) Michel, R.; Mischler, N.; Azambre, B.; Finqueneisel, G.; Machnikowski, J.; Rutkowski, P.; Zimny, T.; Weber, J. V. *Miscanthus x giganteus* straw and pellets as sustainable fuels and raw material for activated carbon. *Environ. Chem. Lett.* **2006**, *4*, 185–189.
(10) Salvadó, J.; Velásquez, J. A.; Ferrando, F. Binderless fiberboard from steam exploded *Miscanthus sinensis*: Optimization of pressing and pretreatment conditions. *Wood Sci. Technol.* **2003**, *37*, 279–286.
(11) Villaverde, J. J.; Domingues, R. M. A.; Freire, C. S. R.; Silvestre, A. J. D.; Pascoal Neto, C.; Ligero, P.; Vega, A. *Miscanthus x giganteus* extractives: A source of valuable phenolic compounds and sterols. *J. Agric. Food Chem.* **2009**, *57*, 3626–3631.
(12) Villaverde, J. J.; Li, J.; Ek, M.; Ligero, P.; Vega, A. Native lignin structure of *Miscanthus x giganteus* and its changes during acetic and formic acid fractionation. *J. Agric. Food Chem.* **2009**, *57*, 6262–6270.
(13) Ye, D.; Montane, D.; Farriol, X. Preparation and characterisation of methylcelluloses from *Miscanthus sinensis*. *Carbohydr. Polym.* **2005**, *62*, 258–266.
(14) Yuan, J. S.; Tiller, K. H.; Al-Ahmad, H.; Stewart, N. R.; Stewart, C. N. Plants to power: Bioenergy to fuel the future. *Trends Plant Sci.* **2008**, *13*, 421–429.
(15) Caridad, R.; Ligero, P.; Vega, A.; Bao, M. Formic acid delignification of *Miscanthus sinensis*. *Cellul. Chem. Technol.* **2004**, *38*, q235–244.
(16) Ligero, P.; Vega, A.; Bao, M. Acetosolv delignification of *Miscanthus sinensis* bark. Influence of process variables. *Ind. Crops Prod.* **2005**, *21*, 235–240.
(17) Villaverde, J. J.; Ligero, P.; Vega, A. Formic and acetic acid as agents for a cleaner fractionation of *Miscanthus x giganteus*. *J. Cleaner Prod.* **2010**, *18*, 395–401.
(18) Ligero, P.; Vega, A.; Villaverde, J. J. Delignification of *Miscanthus x giganteus* by the Milox process. *Bioresour. Technol.* **2010**, *101*, 3188–3193.
(19) Villaverde, J. J.; Ligero, P.; Vega, A. Bleaching *Miscanthus x giganteus* Acetosolv pulps with a new totally chlorine-free sequence and the effect of carbohydrate protectors. *Ind. Eng. Chem. Res.* **2009**, *48*, 9830–9836.
(20) Villaverde, J. J.; Ligero, P.; Vega, A. Bleaching *Miscanthus x giganteus* Acetosolv pulps with hydrogen peroxide/acetic acid. Part 1: Behaviour in aqueous alkaline media. *Bioresour. Technol.* **2009**, *100*, 4731–4735.
(21) del Río, J. C.; Gutiérrez, A.; González-Vila, F. J.; Martín, F.; Romero, J. Characterization of organic deposits produced in the kraft pulping of *Eucalyptus globulus* wood. *J. Chromatogr., A* **1998**, *823*, 457–465.
(22) Freire, C. S. R.; Silvestre, A. J. D.; Pereira, C. C. L.; Neto, C. P.; Cavaleiro, J. A. S. New lipophilic components of pitch deposits from an *Eucalyptus globulus* ECF bleached kraft pulp mill. *J. Wood Chem. Technol.* **2002**, *22*, 55–66.
(23) Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P. Lipophilic extractives in *Eucalyptus globulus* kraft pulps. Behaviour during ECF bleaching. *J. Wood Chem. Technol.* **2005**, *25*, 67–80.
(24) Gutiérrez, A.; del Río, J. C.; González-Vila, F. J.; Martín, F. Analysis of lipophilic extractives from wood and pitch deposits by solid-phase extraction and gas chromatography. *J. Chromatogr., A* **1998**, *823*, 449–455.
(25) Silvestre, A. J. D.; Pereira, C. C. L.; Pascoal Neto, C.; Evtuguin, D. V.; Duarte, A. C.; Cavaleiro, J. A. S.; Furtado, F. P. Chemical composition of pitch deposits from an ECF *Eucalyptus globulus* bleached kraft pulp mill: Its relationship with wood extractives and additives in process streams. *Appita J.* **1999**, *52*, 375–381.
(26) Jansson, M. B.; Worman, P.; Dahlman, O. Reactions of wood extractives during ECF- and TCF-bleaching of kraft pulp. *Pulp Pap. Can.* **1995**, *96*, J42–45.
(27) McCubbin, N.; Folke, J. Significance of AOX vs unchlorinated organics. *Pulp Pap. Can.* **1995**, *96*, J43–J48.
(28) Stauber, J.; Gunthorpe, L.; Woodworth, J.; Munday, B.; Krassol, R.; Simon, J. Comparative toxicity of effluents from ECF and TCF bleaching of eucalyptus kraft pulps. *Appita J.* **1998**, *49*, 184–188.
(29) Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P. Identification of new hydroxy fatty acids and ferulic acid esters in the wood of *Eucalyptus globulus*. *Holzforschung* **2002**, *56*, 143–149.
(30) Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P.; Cavaleiro, J. A. S. Lipophilic extractives of the inner and outer barks of *Eucalyptus globulus*. *Holzforschung* **2002**, *56*, 372–379.
(31) Minor, J. L. Production of unbleached pulp. In *Pulp Bleaching - Principles and Practice*; Dence, C. W., Reeve, D. W., Eds.; Tappi Press: Norcross, GA, 1996; pp 25−57.
(32) Reeve, D. W. Introduction to the principles and practice of pulp bleaching. In *Pulp Bleaching - Principles and Practice*; Dence, C. W., Reeve, D. W., Eds.; Tappi Press: Norcross, GA, 1996; pp 1−24.
(33) Marques, G.; del Río, J. C.; Gutiérrez, A. Lipophilic extractives from several nonwoody lignocellulosic crops (flax, hemp, sisal, abaca) and their fate during alkaline pulping and TCF/ECF bleaching. *Bioresour. Technol.* **2010**, *101*, 260–267.
(34) Silvestre, A. J. D.; Pereira, C. C. L.; Pascoal Neto, C.; Evtuguin, D. V.; Cavaleiro, J. A. S. An easy assessment to the composition of low-molecular-weight organic components of industrial *Eucalyptus globulus* kraft black liquor. *J. Pulp Pap. Sci.* **2000**, *26*, 421–427.
(35) Niemelä, K. Low-molecular weight organic compounds in birch kraft black liquor. Ph.D. Thesis, Helsinki University of Technology, Helsinki, Finland, 1990.

Received for review March 27, 2010. Revised manuscript received June 17, 2010. Accepted June 18, 2010. J. J. Villaverde thanks Fundação para a Ciência e a Tecnologia (FCT) for the award of a postdoctoral grant.