CHEMICAL CHARACTERIZATION OF TAGASASTE (CHAMAECYTISUS PROLIFERUS SPP. PALMENSIS) FIBERS AND THEIR FATE AFTER ORGANOSELV PULPING

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ABSTRACT. The chemical characterization of tagasaste fibers (Chamaecytisus proliferus spp. palmensis) and the fate of their main constituents after organosolv pulping were studied, with especial emphasis in lignin and lipophilic extractives. Py-GC/MS of fibers and pulp showed a lignin with a S/G molar ratio of 1.7 and 1.3, respectively, indicating minor changes in the lignin composition during organosolv pulping. The main lipids identified by GC/MS of extracts from tagasaste fibers were series of fatty acids, α-hydroxy acids, sterols and steroid ketones. Other compounds, such as esters of p-hydroxycinnamic acids (ferulic and p-coumaric acids) and sterol glycosides were also found in minor amounts. The organosolv pulp showed much lower amounts of lipids than the fiber, being mostly fatty acids. The rest of lipophilic compounds were removed to a great extent during the pulping process.

I. INTRODUCTION

Tagasaste (Chamaecytisus proliferus spp. palmensis) is a hardy leguminous and fast-growing shrub of the Fabaceae (Genistae) family cultivated in Australia, New Zealand and Spain (Canary Islands). The shrub is being exploited for high-protein fodder to maintain livestock. The shrub must be grazed with regularity to encourage the formation of bushes with multiple stems, which leads to a high accumulation of residues. These residues have been recently explored as an alternative raw material for pulp production through organosolv pulping [1]. In this work, we have performed a thorough chemical characterization of the tagasaste fibers, with especial emphasis in the study of lignin and lipophilic extractives, and the behavior and structural modifications of these fractions during the organosolv pulping process. The knowledge of the chemical composition of the main components of nonwood plants and their behavior in pulping will be useful for a better utilization of nonwood plants.

The chemical characterization of the lipophilic extractives was performed by GC an GC/MS by using high temperatures short and medium length capillary columns, respectively. This method enables the elution and analysis of intact high molecular weight lipids [2]. The lignin in tagasaste fibers and pulp was characterized using analytical pyrolysis coupled to gas chromatography/mass spectrometry (Py-GC/MS), a powerful analytical tool for the rapid analysis of complex polymer mixtures including lignocellulosic materials [3].

II. EXPERIMENTAL

Samples. Tagasaste fibers and their organosolv pulp were supplied by University of Huelva, Spain. The characteristics of pulp are as follows: cooking temperature, 170º, cooking time, 45 min and ethanol concentration, 40%. The dried samples were milled using a knife mill. For the isolation of lipids, the milled samples were extracted with acetone in a Soxhlet apparatus for 8 h. The acetone extracts were evaporated to dryness and resuspended in chloroform for chromatographic analysis of the lipophilic fraction. For Klason lignin content estimation, the samples extracted with acetone were subsequently extracted with hot water (3 h at 100 ºC). Klason lignin was estimated as the residue after sulfuric acid hydrolysis of the pre-extracted material. Ash content was estimated as the residue after 6 h at 575 ºC.

GC and GC/MS Analyses. The GC analyses of the extracts were performed in an Agilent 6890N GC system using a short-fused silica capillary column (DB-5HT, 5 m × 0.25 mm I.D., 0.1 µm film thickness). The temperature program was started at 100 ºC with a 1-min hold and then raised to a final temperature of 350 ºC at 15 ºC/min, and held for 3 min. The injector and flame-ionization detector temperatures were set at 300 and 350 ºC, respectively. Helium was used as the carrier gas at a rate of 5 mL/min, and the injection was performed in splitless mode.

The GC/MS analyses were performed with a Varian model Star 3400 GC equipped with an ion trap detector (Varian Saturn 2000) using a medium-length (12 m) capillary column of the same characteristics described above. The oven was heated from 120 (1 min) to 380 ºC at 10 °C/min and held for 5 min. The transfer line was kept at 300 °C. The injector was temperature programmed from 120 (0.1 min) to 380 ºC at a rate of 200 °C/min and held until the end of the analysis. Helium was used as the carrier gas at a rate of 2 mL/min. Trimethylsilyldiazomethane methylation and bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation, in the presence of pyridine, were used to produce the appropriate derivatives, when required.

Py-GC/MS. The pyrolysis of tagasaste fibers (1 mg) was performed in a micro-furnace pyrolyzer (model 2020, Frontier Laboratories Ltd) directly connected to a GC/MS system Agilent 6890 equipped with a fused
silica capillary column HP 5MS (30 m × 0.25 mm × 0.25 μm). The detector consisted of an Agilent 5973 mass selective detector. The pyrolysis was performed at 500 °C. The final temperature was achieved at a rate of 20 °C/min. The GC/MS conditions were as follows: oven temperature was held at 50 °C for 1 min and then increased up to 100 °C at 30 °C/min, from 100 to 300 °C at 10 °C/min and isothermal at 300 °C for 10 min using a heating rate of 20 °C/min in the scan mode. The carrier gas used was helium with a controlled flow of 1 ml/min.

III. RESULTS AND DISCUSSION

The tagasaste fibers are characterized by a lignin content of 16.6%, estimated as Klason lignin. This value is higher than that of other nonwood fibers, such as hemp or flax but lower than in wood [4]. The content of lipids was 1.4%, higher than hemp but lower than that of other nonwood materials used for papermaking, such as flax [5]. The composition of tagasaste organosolv pulp is characterized by a lower lignin and extractives content, 11% and 0.3%, respectively. Other authors [6] have reported that under the conditions of ethanol/water pulping, hemicelluloses are rapidly hydrolyzed and solubilized but the cellulose is relatively untouched. As a consequence, these pulps display a high glucan (cellulose) level, in agreement with the present holocellulose result (78.0% in fibers and 86.4% in pulp).

The lignin in the tagasaste fibers and pulp were characterized in situ by Py-GC/MS, as shown in Figure 1. The Py-GC/MS analysis of the fibers released predominantly compounds arising from carbohydrates (67%) and minor amounts of lignin-derived phenols (34%). The lignin-derived phenols arise from guaiacyl (G) and syringyl (S) lignin units, with a predominance of the S units (S/G molar ratio of 1.7). The main lignin-derived compounds identified were 4-vinylsyringol (38), syringol (24), 4-methylsyringol (29), 4-ethylsyringol (35) and 4-vinylguaiacol (23). Syringaldehyde (41), syringylacetone (47) and trans-sinapaldehyde (49) were also identified. The Py-GC/MS analysis of pulp shows only a small decrease in the S/G ratio after pulping (1.3), which indicates that the lignin composition has not been largely modified, in contrast to what occurs during alkaline pulping that preferentially attacks and removes the S-lignin units.

The GC/MS chromatograms of the lipids from tagasaste fibers, as well as the lipids in the pulp, are shown in **Figure 2**. The identities and abundances of the main lipid classes identified are summarized in **Table 1**. The main lipids identified in tagasaste fibers were series of fatty acids, α-hydroxy acids, sterols and steroid ketones. The sterols include sitosterol, stigmasterol, stigmastanol and campesterol. Other compounds, such as esters of p-hydroxycinnamic acids (ferulic and p-coumaric acids) and sterol glycosides were also found in minor amounts. On the other hand, the organosolv pulp showed much lower amounts of lipids, being mostly fatty acids. The rest of lipophilic compounds were removed to a great extent during the pulping process.

**Figure 2.** GC/MS of the lipophilic extracts from tagasaste fibers and their Organosolv pulp. Key labels are: 1: α-tocopherol, 2: campesterol, 3: stigmasterol, 4: sitosterol, 5: stigmastanol, 6: 7-oxocampesterol, 7: 7-oxostigmasterol, 8: 7-oxositosterol, FAₙ: fatty acids.
Table 1. Composition of lipids (mg/Kg) from tagasaste fibers and their organosolv pulp.

<table>
<thead>
<tr>
<th>compound</th>
<th>Tagasate fibers</th>
<th>Organosolv pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>steroid hydrocarbons</td>
<td>30.1</td>
<td>1.7</td>
</tr>
<tr>
<td>n-alkanes</td>
<td>9.6</td>
<td>7.7</td>
</tr>
<tr>
<td>fatty acids</td>
<td>141.8</td>
<td>25.1</td>
</tr>
<tr>
<td>α-hydroxy fatty acids</td>
<td>111.4</td>
<td>0.0</td>
</tr>
<tr>
<td>sterols</td>
<td>101.9</td>
<td>0.6</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>2.1</td>
<td>0.1</td>
</tr>
<tr>
<td>sterol esters</td>
<td>28.3</td>
<td>0.0</td>
</tr>
<tr>
<td>steryl glycosides</td>
<td>13.2</td>
<td>0.0</td>
</tr>
<tr>
<td>steroid ketones</td>
<td>30.5</td>
<td>1.1</td>
</tr>
<tr>
<td>esters of p-hydroxycinnamic acids</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>monoglycerides</td>
<td>8.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

IV. CONCLUSIONS
The present work has shown the behavior of lignin, lipids and cellulose from the tagasaste fibers during organosolv pulping (ethanol/water cooking). The study of the chemical composition of tagasaste fibers show that the extractives and lignin contents encourage the use of this species as a suitable source of fiber. While organosolv pulping does not affect the structure of the lignin, as shown by the S/G ratio, the lipids have been removed to a high extent.

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VI. REFERENCES