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RELATIONSHIPS BETWEEN MICRO-FIBRILLAR ANGLE, MECHANICAL PROPERTIES AND BIOCHEMICAL COMPOSITION OF FLAX FIBERS

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ABSTRACT
An elementary plant fiber could be assimilated to a laminate, mainly constituted of the secondary wall S2 layer, made of a few non-crystalline polysaccharides reinforced by cellulose fibrils organized in a helix, with a microfibrillar angle (MFA) around 10° relative to the longitudinal fiber axis. This paper investigates the relationships between the MFA, the mechanical properties and the biochemical composition of different varieties of flax. To conduct this study, tensile tests on elementary flax fibers, X-ray diffraction, and solvent extractions have been carried out. Within the different varieties of flax, Young’s modulus was found to be negatively correlated with the MFA. The results showed that the ratio between hemicelluloses (matter extracted with alkali) and pectins (hydrolyzed with acids) is highly correlated with the tensile properties; concurrently, we showed the great influence of pectic acids on the fiber’ Young’s modulus, and on the orientation of the microfibrils.

INTRODUCTION
The depletion of our natural resources, as well as the increasing impacts of our society on our environment, suggest that there is a need for new research in the design of composite materials. Thus, for many industrial products, plant fibers could be used as a substitute for synthetic fibers in composite materials reinforcement. In Europe, the most interesting are flax and hemp fibers, due to their high-performance specific mechanical properties [1], their environmental and ecological benefits [2], as well as their low cost.

Flax or hemp fibers exhibit a complex, hierarchical and multi-component structure; they could be considered, at their scale, as complex composite materials. Flax fibers commonly have a polygonal section, and are arranged into concentric cell-wall layers with a channel in the middle, called the lumen, which corresponds to the remains of the cytoplasm and nucleus. As a sink, it may play a major role in the water sorption of the material [3]. The bulk of the fiber cell walls is constituted by the secondary wall, and is divided into 3 different layers - S1 (0.5-2 µm thick), S2 (5-10µm) and S3 (0.5-1µm)- and provides the reinforcement of the plant
structure. The average diameter of a flax fiber is around 15 µm [4]. The main layer, representing around 80% of the total section, is the S2 layer, constituted of highly crystalline cellulose fibrils spirally wound in a matrix of amorphous hemicelluloses and pectins [5]; the cellulose fibrils make a 10° angle with the axis of the fiber [6]- which is called micro-fibrillar angle (MFA)- and their crystallinity is about 65% for the secondary cell wall fibrils [7].

Due to this multi-component structure, plant fibers have a complex mechanical behavior [8]; after a first zone corresponding to the beginning of the fiber loading, their stress strain curve exhibits a non-linear part probably induced by the initiation of the reorientation of cellulose fibrils in the secondary wall (S2). As assumed by Baley [8], the non-linearity that is noticed at the beginning of a stress-strain tensile curve may be associated to the realignment of cellulose micro-fibrils. Some recent works corroborated this hypothesis by pointing out that the micro-fibrillar realignment during a tensile loading was not complete for hemp [9] or flax [10]. According to Hearle [11], this reorientation is caused by the shearing of the polysaccharide network during the loading; this loading induces an increase in the cellulose fibrils’ length as well as in the inter-fibrillar amorphous areas. The second region of the loading curve appears linear, a typical characteristic for an elastic behavior. At this particular moment, the cellulose fibrils are probably more aligned in the direction of the tensile axis.

In flax cell walls, the cellulose macro fibrils are embedded in an amorphous polysaccharide matrix composed mainly of pectins and hemicelluloses. The hemicelluloses not only act as a matrix but also, thanks to a structure similar to that of cellulose, may act as a coupling agent [8, 12]; its nature varies according to whether it is in the primary layer of fibers (e.g. xyloglucan) or in the secondary layer (xylanes and glucomananes). Alix et al. [3] compared the structure and the mechanical properties of oleaginous (Oliver) and textile (Hermes) flax. They explained the high mechanical properties of textile flax by the inter-fibrillar distances (lower for Hermes) due to lower amount of non-cellulosic polymer matrix between these cellulose fibrils. In the case of Hermes flax, the presence of glucomannan bridges could explain part of the stiffness differences.

As underlined before in this paper, the MFA has an important influence on the mechanical behavior of plant fibers, and it could be considered as an important structural parameter. This MFA could differ according to the species under study. Thus, Salmen [13] compared the MFA values of various wood fibers with their associated mechanical properties. The MFA could be approached through microscopic methods (Scanning Electronic Microscopy (SEM) or Atomic Force Microscopy (AFM)) but, due to the low dimensions of the studied areas, it is very difficult to obtain an absolute MFA value, at the scale of the fiber. A few research teams have used X-ray diffraction to understand the influence of the MFA on the mechanical properties of plant fibers or wood [14]. The first X-ray investigations on plant fibers are attributed to Meyer [15] on ramie; they calculated the crystalline modulus of the cellulose under loading from the 040 diffraction plane. Cave [16] showed, as far as wood is concerned, that the intensity profiles of 040 planes make it possible to obtain a direct measure of the MFA distribution into the S2 layer.

The purpose of this work is to contribute to the knowledge of the impact of the plant fibers micro-structure, and the biochemical composition on their mechanical properties. We selected various oleaginous and textile flax fiber varieties representing a range of mechanical behaviors. First, we determined the fibers’ MFA by using X-ray diffraction before investigating their biochemical composition. The paper closes on a discussion about the interactions and correlations between the mechanical, structural and biochemical results.
EXPERIMENT

FLAX FIBER

Table 1 shows the different flax varieties used for this study.

<table>
<thead>
<tr>
<th>Flax variety</th>
<th>Type of flax</th>
<th>Culture year</th>
<th>Culture Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermes</td>
<td>Textile</td>
<td>2003</td>
<td>France (Normandy)</td>
</tr>
<tr>
<td>Ariane</td>
<td>Textile</td>
<td>2002</td>
<td>France (Normandy)</td>
</tr>
<tr>
<td>Agatha</td>
<td>Textile</td>
<td>2003</td>
<td>France (Normandy)</td>
</tr>
<tr>
<td>Everest (E5)</td>
<td>Oleaginous</td>
<td>2005</td>
<td>France (Brittany)</td>
</tr>
<tr>
<td>Everest (E8)</td>
<td>Oleaginous</td>
<td>2008</td>
<td>France (Brittany)</td>
</tr>
<tr>
<td>Alaska</td>
<td>Oleaginous</td>
<td>2006</td>
<td>France (Brittany)</td>
</tr>
<tr>
<td>Hivernal</td>
<td>Oleaginous</td>
<td>2006</td>
<td>France (Brittany)</td>
</tr>
<tr>
<td>Oliver</td>
<td>Oleaginous</td>
<td>2003</td>
<td>France (Brittany)</td>
</tr>
</tbody>
</table>

Table 1: Flax varieties used for the study

After being pulled, plants were laid over the field for 4 weeks to allow stem drying and dew-retting. Retting consists in the development of fungi within the stems, which degrades their cortical tissues, and further facilitates the extraction of the fibers. The retted plants were further scutched to extract what we designated as technical fibers, which consisted of a partially divided bundle (diameter range 60-80 µm) containing 15 or more elementary fibers. All the selected fibers were manually extracted in the middle of the stem.

TENSILE TESTS ON ELEMENTARY FLAX FIBERS

The fiber was clamped on a universal MTS type tensile testing machine equipped with a 2 N capacity load cell and loaded at a constant crosshead displacement rate of 1 mm/min up to rupture. The determination of the mechanical properties was made in accordance with the NFT 25-704 standard which takes into account the compliance of the loading frame. For each kind of fiber, at least 50 fibers were tested.

ESTIMATION OF FLAX FIBER MICRO-FIBRILLAR ANGLES BY X-RAY DIFFRACTION

An X-ray diffractometer from Oxford diffraction (Supernova model) was used to estimate the micro-fibril angle. The Supernova system consists of a kappa geometry, four circle goniometry for sample orientation with a detector arm, a CCD area detector delivering a digitized signal to 18-bit resolution and an X-ray tube, with a copper source, the nominal point of which is at 50 keV/1 mA. At the sample position, the beam diameter is about 300 µm. The sample can be observed with a video microscope for accurate positioning. Fiber bundle of about 100 µm in diameter were used. In order to estimate the micro-fibril angle for elementary flax fiber bundle, a four-steps methodology is used:

- Calibration of the coordinates with a crystal powder
- Estimation of the 002 optimal circle
- Extracting the diffraction pattern
- Micro fibrillar angle identification by curve fitting

The MFA is then deduced from the parameters of the fitted function according to Cave’s equation [16]. The exposure time was set to 60 seconds in order to get the optimal ration signal/ noise. Four images of 60 seconds were used for the dark noise subtraction.
EXTRACTION OF POLYSACCHARIDES AND SUGAR ANALYSIS

In order to divide the bundles into elementary fibers, the fiber bundles were pre-treated at 100°C (3 x 2h) with water, and then with EDTA-Na$_2$. On the whole, 13.0 ± 0.5% to 20 ± 2% of dry matter was removed from the technical fibers of, respectively fiber flax and oleaginous varieties. The difference between the two types of flax is mainly originated from the largest stem section of the oleaginous varieties with large amount of cortical tissues to be degraded. The cleaned fibers were then successively treated with 0.02 M HCl and 1.5 M NaOH/100mM NaBH$_4$ (once 1h 100°C+ twice in H$_2$O for 1h at 100°C) in order to release the polysaccharides (named EH and EOH, respectively) that encrusted (EH) and coated (EOH) the cellulose macrofibrils [17]. Although EOH might contain pectic polymers [3], they were sometimes referred to as hemicelluloses, because of the alkali treatment. Three independent series were run for each sample. Total sugars and galacturonic acids were colorimetrically assayed [18-19].

RESULTS AND DISCUSSION

IMPACT OF THE FLAX VARIETY ON THE MECHANICAL PROPERTIES OF FLAX FIBERS

Table 2 reports the Young’s modulus, strength and elongation at break of the flax fibers for the different flax varieties that were studied. Most varieties had been studied by our research team in previous works [4, 8, 17, 20]. The Oliver variety has been characterized during this work. In addition to these flax fibers, the mechanical properties of hemp fibers [21] are indicated.

First, we can notice some important mechanical properties variations between the different flax fibers. In general, the properties of the textile-type varieties were higher (although not significantly so) than oleaginous-type varieties. For two varieties of the same type, cultivated the same year in the same geographic area, therefore probably submitted to the same climatic conditions, important mechanical property differences can be noticed. This is the case for Hermes and Agatha 2003, or Alaska and Hivernal 2006. These observations indicate that the genetic pool of each type and variety probably has some influence on their mechanical properties. Altogether, the lowest tensile properties were typical of some varieties of the oleaginous type, but the high values of Everest 2008 and Hivernal 2006 showed the possibility of high values for the oleaginous type. We might hypothesize that oleaginous type fibers were more sensitive to the impact of bad growth conditions.

<table>
<thead>
<tr>
<th>Fibers</th>
<th>Number of fibers</th>
<th>Average diameter (mm)</th>
<th>Young Modulus (GPa)</th>
<th>Strength at break (MPa)</th>
<th>Elongation at break (%)</th>
<th>AMF (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermes 2003</td>
<td>37</td>
<td>19.6 ± 6.7</td>
<td>68.2 ± 35.8</td>
<td>1454 ± 835</td>
<td>2.3 ± 0.6</td>
<td>8.6 ± 0.2</td>
</tr>
<tr>
<td>Ariane 2002</td>
<td>77</td>
<td>23.0 ± 5.7</td>
<td>54.1 ± 15.1</td>
<td>1339 ± 486</td>
<td>3.3 ± 0.8</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>Agatha 2003</td>
<td>45</td>
<td>21.3 ± 6.3</td>
<td>57.0 ± 29.0</td>
<td>865 ± 413</td>
<td>1.8 ± 0.7</td>
<td>9.3 ± 0.4</td>
</tr>
<tr>
<td>Everest 2005</td>
<td>9</td>
<td>16.9 ± 4.9</td>
<td>41 ± 12.5</td>
<td>663 ± 307</td>
<td>1.8 ± 0.4</td>
<td>8.9 ± 0.1</td>
</tr>
<tr>
<td>Everest 2008</td>
<td>30</td>
<td>15.4 ± 5.1</td>
<td>75 ± 21.6</td>
<td>1232 ± 554</td>
<td>2.1 ± 0.8</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td>Alaska 2006</td>
<td>20</td>
<td>15.3 ± 5.4</td>
<td>46.3 ± 12.1</td>
<td>691 ± 253</td>
<td>1.8 ± 0.6</td>
<td>9.5 ± 0.1</td>
</tr>
<tr>
<td>Hivernal 2006</td>
<td>23</td>
<td>12.9 ± 3.3</td>
<td>67.5 ± 23.7</td>
<td>1119 ± 490</td>
<td>1.9 ± 0.5</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>Oliver 2003</td>
<td>78</td>
<td>17.5 ± 3.6</td>
<td>47.2 ± 21.3</td>
<td>751 ± 414</td>
<td>1.7 ± 0.6</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>Hemp</td>
<td>50</td>
<td>25.4 ± 6.0</td>
<td>19.1 ± 11.3</td>
<td>685 ± 590</td>
<td>2.5 ± 1.1</td>
<td>11.2 [9]</td>
</tr>
</tbody>
</table>

Table 2: Mechanical properties of elementary plant fibers.

From one year to the next, opposite weather conditions can be observed, which could induce important mechanical property gaps, as evidenced with the Everest 2005 (E5) and 2008 (E8) varieties. Some particular weather conditions (severe drought, for example) could induce
Hydric stress, which can be prejudicial to the development and the maturation of flax fibers. In the case of Everest, recent works [4] showed that the 2005 variety (E5) has an slightly higher average diameter than those of the other culture years. This result seems to be correlated with very low yields of seed obtained in 2005 which, as noted above, indicated that the maturity of E5 was lower than that of E8. The high average diameters might be related to the lack of fiber structuring which occurred during the last step of seed maturation [12]; generally, mechanical properties and average diameter are correlated and higher mechanical properties are found for lower diameters [8].

In the second part of this experimental section, we focus on the determination of the MFA fibers by using X-ray diffraction.

**Use of the X-ray Diffraction to Estimate the Flax Fiber MFA**

Table 2 reports the MFA of the different flax varieties under study. The MFA values ranged between 8.3 and 9.5° according to the variety of flax fiber. The micro-fibrillar values that were obtained correlate well with those of the literature. There are few papers about direct experiments on flax fibers; Astley et al. [22], by using X-ray diffraction obtained MFA around 15° for wet flax fibers and 11° for the dry same fibers. Some authors report MFA values around 10° for flax fibers [6], which is well correlated with our results. However, our MFA results have to be tempered due to the scale measurement. The XRD apparatus does not make it possible to estimate orientation on an elementary cell; it could be interesting, in forthcoming work, to carry out new experiments, at low scale, in order to obtain information at the cell wall scale. These investigations could be performed on a Synchrotron machine, which exhibits a very low spot size suitable for elementary fibers.

**Biochemical Composition of the Flax Studied Varieties**

Table 3 presents the cell wall composition of the flax fibers. First, we can notice that the sum of EH and EOH relative percentages were higher in oleaginous (19.9 ± 1.8) than in textile (15.4 ± 1.0) varieties. Conversely, the cellulosic residue (RC) was higher in textile flax than in oleaginous varieties. As shown in the simplified figure 1, EH moieties would correspond to the secondary wall pectins, while EOH matter mainly represented hemicelluloses, coating with the micro-fibrils, and showing various conformations.

<table>
<thead>
<tr>
<th>Fibers</th>
<th>∆m EH (%)</th>
<th>∆m EOH (%)</th>
<th>∆m EOH / ∆m EH</th>
<th>RC (%)</th>
<th>UA EH (mg/mg)</th>
<th>UA EOH (mg/mg)</th>
<th>UA' EH (mg/gF)</th>
<th>UA' EOH (mg/gF)</th>
<th>UA' EOH/EH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermes 2003</td>
<td>6.3</td>
<td>9.8</td>
<td>1.6</td>
<td>83.9</td>
<td>0.12</td>
<td>0.07</td>
<td>7.8</td>
<td>6.9</td>
<td>0.89</td>
</tr>
<tr>
<td>Ariane 2002</td>
<td>5.0</td>
<td>8.8</td>
<td>1.8</td>
<td>86.2</td>
<td>0.18</td>
<td>0.08</td>
<td>10.6</td>
<td>7.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Agatha 2003</td>
<td>7.0</td>
<td>9.3</td>
<td>1.3</td>
<td>83.6</td>
<td>0.12</td>
<td>0.07</td>
<td>8.4</td>
<td>6.4</td>
<td>0.76</td>
</tr>
<tr>
<td>Everest 2005</td>
<td>9.7</td>
<td>10.5</td>
<td>1.1</td>
<td>79.8</td>
<td>0.11</td>
<td>0.06</td>
<td>10.5</td>
<td>6.1</td>
<td>0.58</td>
</tr>
<tr>
<td>Everest 2008</td>
<td>6.2</td>
<td>12.2</td>
<td>2.0</td>
<td>81.6</td>
<td>0.12</td>
<td>0.06</td>
<td>7.2</td>
<td>7.9</td>
<td>1.09</td>
</tr>
<tr>
<td>Hivernal 2006</td>
<td>10.5</td>
<td>13.6</td>
<td>1.3</td>
<td>75.9</td>
<td>0.08</td>
<td>0.05</td>
<td>8.3</td>
<td>7.2</td>
<td>0.87</td>
</tr>
<tr>
<td>Oliver 2003</td>
<td>10.8</td>
<td>6.0</td>
<td>0.6</td>
<td>83.2</td>
<td>0.09</td>
<td>0.07</td>
<td>9.3</td>
<td>4.1</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Table 3: Cell-wall composition of fibers.

As reported cell wall models [23-24] they could be linearly arranged along the micro-fibrils, or form bridges, loops or free chains, inducing main differences into the cell wall stiffness. As indicated by Alix et al. [3], EOH also contained structural pectins ensuring some kind of interphase between the cellulosic micro fibrils and the matrix pectins (EH). Thus, oleaginous
Oliver and textile Hermes flaxes were shown to exhibit important differences in their cell wall structures, mainly in the amount of EH and ratio of pectic acid between EOH and EH.

![Figure 1: Schematic representation of biochemical arrangement of the flax fiber S2 layer.](image)

Secondly, when the galacturonic acids (UA) were expressed in mg per mg of extract (UA EH\textsuperscript{1} & UA EOH\textsuperscript{1}), the values in the textile-type fibers were slightly higher than in oleaginous-type ones, i.e. 0.14 ± 0.01 / 0.10 ± 0.01 in EH, and 0.073 ± 0.005 / 0.061 ± 0.004 in EOH. On the other hand, when expressed per 1g of pre-treated fiber (UA' EH\textsuperscript{2} & UA' EOH\textsuperscript{2}), the average values were similar in both type of flax, due to the largest amount of extracted matter in oleaginous type. These UA in EH were mainly associated to S2 cell wall matrix pectins whereas those present in EOH corresponded to more structural pectins. The ratio UA’ EOH/OH\textsuperscript{3} quantifies the relative content of structural pectins. The average values were similar in both types of flax. When taken together, the number of micro mol of UA per g of fibers accounted for 77 ± 3, which is characteristic of well retted fibers.

DISCUSSION AND INTERPRETATIONS

Table 4 shows the correlations between the biochemical composition and the MFA values with the mechanical properties obtained on elementary flax fibers. The \(r^2\) values are calculated considering the hypothesis of a linear correlation. The (-) sign corresponds to a negative slope.

First, a negative correlation can be noticed between EH and the stress at break. The more important the EH quantity, the larger is the interfibrillar space absorbing the tensile stress, which allowed for more sliding between macro-fibrils. Moreover, the EOH/EH ratio is positively related to all the tensile parameters. This result confirms the importance of the EOH/EH ratio which conditions the mechanical performances of the cell walls, which as was previously hypothesized by Alix et al [25] when studying only two varieties.

The percentage of cellulosic residue was not correlated with the mechanical parameters, but was slightly so with the strain at break. Nevertheless, due to the great mechanical properties of the cellulose (around 135 GPa) [26], the fiber cellulose rate (measured between 75.9 and 86.2%) is large enough to confer high mechanical properties to flax fibers. Thus, if basal values of tensile parameters would be insured by the high rate of cellulose, the variations between minimal and maximal values were mainly due to the structuring and detailed organization of the cell walls. This hypothesis is reinforced by the importance of the EOH/EH ratio which quantifies the structuring components part into the S2 layer. Moreover, improvements into mechanical properties and Young’s Modulus accommodation noticed after cyclic solicitations [21] showed that micro-fibrils reorientation and changes into cell wall structuring have a real influence on the fiber mechanical properties.
A positive correlation has been found, especially in EH, between the UA percentage (relative to the mass of polysaccharides) and the strain at break. The more important the UA quantity in the matrix, the higher the sliding between micro-fibrils during the tensile solicitation. On the other hand, UA’ in EOH was positively related with strength and, more interestingly, with E, while UA’ in EH was negatively related with E. As evidenced in figure 6, the ratio UA’ EOH/EH showed the highest positive correlation with the tensile module ($r^2 = 0.85$). If we omitted the not completely mature Everest 2005 fiber [4], the $r^2$ value would reach the extreme value of 0.96. It was also worth observing that MFA was highly negatively related to this ratio.

Finally, an interesting relationship was found between the MFA and the flax fiber mechanical properties, as it exists for wood but within a much wider range of MFA values [13]. A strong negative correlation exists between the MFA and the flax fibers Young’s modulus ($r^2 = -0.75$). This correlation is moderate with the tensile strength at break ($r^2 = -0.37$) and the strain at break ($r^2 = 0.23$).

As explained in the experimental section, micro-fibrillar angles have been obtained on non-solicited fiber bundles whereas the tensile Young’s modulus has been calculated on the second part of the stress-strain curve of elementary fibers. This point could be questionable, but the determination of the stiffness in the first part of the stress-strain curve has little meaning due to the progressive loading of the micro fibrils occurring at the beginning of the test; in this case, the values that were obtained would not be representative of the real properties of the flax fibers.

**CONCLUSIONS**

For the first time concerning plant cellulosic fibers, it was shown that the lower the MFA, the higher the Young's modulus. Moreover, this work confirms the importance of the ratio between the amount of coating polymers and that of the matrix pectin on tensile behavior. Importantly, the content of uronic acids was positively correlated with the tensile Young’s modulus, highlighting their structural role in the tensile properties of the flax cell walls. Due to their negative correlation with the MFA, we hypothesize that pectic acids may influence the micro-fibrillar orientation when they are secreted in the secondary wall.
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