Effect of harvest time and field retting duration on the chemical composition, morphology and mechanical properties of hemp fibers

Ming Liu a, Dinesh Fernando b, Geoffrey Daniel b, Bo Madsen c, Anne S. Meyer d, Marcel Tutor Ale a, Anders Thygesen a,∗

a Center for Bioprocess Engineering, Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søltofts Plads 229, 2800 Lyngby, Denmark
b Department of Forest Products/Wood Science, Swedish University of Agricultural Sciences, Vallvägen 9d, 75651 Uppsala, Sweden
c Department of Wind Energy, Technical University of Denmark, Frederiksborgvej 399, 4000 Roskilde, Denmark

Article history:
Received 31 October 2014
Received in revised form 3 February 2015
Accepted 5 February 2015
Available online 16 February 2015

Keywords:
Cannabis sativa L.
Hemp fiber
Fiber extraction
Field retting
Mechanical properties

The large variability in the mechanical properties of hemp fibers is an issue in relation to their use in high-grade composites. The objective of the present study was to determine the optimal growth stage for harvesting hemp fibers for use in composites and to evaluate the effect of field retting time on mechanical performance of the fibers. Reduction in bast content and thickness of the primary bast fiber layer in stems were found to be highly significant (P<0.01) with plant maturity. A significant increase in the secondary fiber fraction occurred with maturity, reaching a maximum value of 10% at seed maturity. A highly significant reduction in cellulose deposition in fiber cell walls was reflected by reduced fiber wall thickness with plant maturity and was related to the development and ripening of hemp seeds. A statistically significant increase in lignin deposition and a slight decrease in pectins in hemp fiber cell walls were also noted with stem maturity. Microscopy observations and histochemical analyzes corroborated the results from the chemical analyzes and revealed variations in morphological aspects and spatial micro-distributions of carbohydrates and lignin within the cell structure of the hemp stems between early- and late growth phases. Fibers harvested at the beginning of flowering exhibited high tensile strength and strain, which decreased with plant maturity. Reduction in strength was related to the increase in proportion of secondary fibers and decrease in cellulose deposition leading to inferior properties of fibers. A negative effect of field retting occurred only after extended field retting (i.e., 70 days) which was presumably due to accelerated degradation of cellulose by the action of microorganisms.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Hemp fibers are cellulose-rich cells that are attractive as reinforcement agents in composite materials due to their low cost and density, good mechanical properties and potential sustainability and biodegradability (Islam et al., 2011; Thygesen et al., 2005, 2011). The hemp fibers suitable for composites are the primary- and secondary fibers (i.e., bast fibers) situated in the cortex of the hemp plant stem. The bast fibers encircle the core xylem and originate from the procambium and correspond to sclerenchyma primary cells (Crönier et al., 2005; Esau, 1943). Their morphological features differ significantly from those of xylem fibers. In addition, the morphology and chemical composition of bast fibers vary with maturity (Charlet et al., 2007; Duval et al., 2011; Mediavilla et al., 2001). This results in large variations in the mechanical properties of the fibers (Placet et al., 2012), and these variations are generally considered a major barrier for using hemp fibers in composites where high reliability and stability of fiber properties are required.

The main chemical components of hemp fiber cell walls are cellulose, hemicelluloses, lignin and pectin and the fibers are bound together by a pectin and lignin-rich middle lamella (ML) (Love et al., 1994; Nykter et al., 2008). For high-grade composites, the ML-fiber bonding must be degraded to obtain individual fibers and/or small fiber bundles. Therefore to increase the ease of fiber extraction from plants and reduce fiber breakage, the stems are normally retted before mechanical separation (termed “decortication”). The retting stage is critical for the broad use of hemp fibers with respect to economic aspects and fiber quality (Keller et al., 2001; Mediavilla et al., 2001).
In field retting (also known as dew-retting), plant stems are spread out in the fields where they are attacked mainly by fungi. The pectinolytic enzymes expressed by the fungi can degrade the pectin in the middle lamella regions between fibers (Henriksson et al., 1997). Field retting is still widely used due to its low cost (Bacci et al., 2010), but is limited to geographic regions where weather conditions are suitable for fungi proliferation. Field retting also causes many problems such as increased scattering of fiber properties, insecurity of fiber supply due to poor weather conditions and may also cause delays in the planting of subsequent crops. Due to these disadvantages, more efficient and controllable methods have been investigated, including chemical treatment (Song and Obendorf, 2006), mechanical defibration (Vignon et al., 1996), and enzymatic retting (Li and Pickering, 2008). However, those methods require high energy input or expensive enzymes and/or may generate costly wastes (Tahir et al., 2011).

For natural fibers, their mechanical performance (e.g., tensile strength) largely depends on a number of crucial physical and chemical parameters, and the mechanical performances of natural hemp fibers were recently shown to be highly dependent on fiber diameter (Duval et al., 2011; Marrot et al., 2013; Placet et al., 2012). Tensile strength is found to decrease as the fiber diameter increases and this diameter dependence is closely related to both the number of fiber defects (i.e., kinks or dislocations) and number of single fibers contained in the fiber bundles (Fan, 2010).

Hemp contains secondary bast fibers situated outside the vascular cambium. These secondary fibers are shorter (approx. 2 mm long) and thinner (approx. 15 μm in diameter) than primary fibers (i.e., tenths of mm in length and 18–24 μm in diameter) (Mishra 2009; Sankari 2000a). According to Amaducci et al. (2008), these secondary fibers are primarily located at the bottom of the plant stem. Their formation has been reported to cause a reduction in both fiber yield and quality after flowering (Mediavilla et al., 2001). Thus, the mechanical properties of hemp fibers are dependent on many parameters such as fiber diameter, defects, chemical composition, and the presence/proportion of secondary fibers.

The aims of the present work were to provide an improved understanding of the reduction of mechanical properties of hemp fibers in relation to morphological features and chemical composition during growth and field retting.

2. Materials and methods

2.1. Raw material

2.1.1. Cultivation and harvest

The hemp (Cannabis sativa L.), variety USO-31, was sown at a rate of 45 kg/ha on May 5th 2013 in France (N 48.85°, E 3.02°) (WCS84) by hemp cultivation companies (Planète Chanvre and Bafa Neu GmbH). The seeds were sown with the seed drill 3–4 inches deep. The hemp plants were fertilized with 80 kg/ha N, 45 kg/ha K and 45 kg/ha P. The monococious hemp plants were harvested at two developmental stages: (1) at the beginning of flowering (i.e., early harvest on July 18th 2013); and (2) seed maturity (i.e., late harvest on Sep 6th 2013). Using the definition of hemp growth stages given by Mediavilla et al. (1998), the two stages selected correspond to codes 2101 and 2204. Precipitation was not well distributed over the season (beginning of May to the end of September, 2013). From sowing date to the early harvest date, the weather was cool with an average temperature of 14.5 °C and precipitation was relatively evenly distributed with a total of 115 mm. Between early and late harvest (first retting period for early harvest sample, 50 days in total), the weather was quite hot (especially during daytime) with an average temperature of 19.6 °C and dry with a precipitation of 52 mm. From late harvest to the end of field retting (20 days in total), the weather was humid with a total precipitation of 41 mm and cool with an average temperature of 14.2 °C (Fig. 1).

2.1.2. Sampling and storage

In this study, sampling involved a complete randomized block design with three replications. With each harvest (i.e., early- and late harvest), in every replicate, 10 m² above-ground part of hemp was harvested and the number of plants was determined. Considering the high dependence of morphological feature, chemical composition and mechanical properties on hemp stem sections, only the bottom section (one third above the base of the stem) of the plant was investigated (Charlet et al., 2007; Duval et al., 2011). A randomized sample of 20 bottom sections of plants per replication was used to determine the diameter of hemp stem and bast content.

A flow diagram of the setup for this study is presented in Fig. 2. For early harvest, a randomized sample of 80 plants per replication was divided into 4 groups (Group 1–Group 4). For late harvest, a randomized sample of 100 plants per replication was divided into 5 groups (Group 1–Group 5).

2.2. Cell wall isolation

The bast fibers were air-dried at 40 °C with an air flow of 150 m²/(m² grid b) (Maskinfabrikken Thisted, Denmark Type 150) and ground with a crushing microfine grinder (JKAs, MF 10.1) to a particle size of 1 mm. Samples of about 3 g were then extracted in a Soxhlet apparatus (Gerhardt EV6 All/16 No. 10-0012) for 5 h using a 300 mL solution of toluene-ethanol-acetone (4:1:1 by volume) (Sluiter et al., 2008; Özmen et al., 2013). For each sample, the final residue was dried at 50 °C for 12 h and the resulting residue was designated as cell wall residue (CWR).

2.3. Chemical analysis of CWR

Chemical analyzes were done using two-step sulfuric acid hydrolysis at 72% and 4% (w/w), according to the method of the US National Renewable Energy Laboratory (Sluiter et al., 2011). After acid hydrolysis, the hydrolysate was collected for monosaccharide analysis. Hemp fibers are characterized by their low lignin content, and the lignin content of hemp bast fibers is usually characterized by Klasen lignin. (Charlet et al., 2007; Gutiérrez et al., 2006). For Klasen lignin analysis, a crucible with filtered solids (i.e., acid-insoluble ash + acid–insoluble lignin) was dried in an oven at 105 °C for 12 h. After cooling to room temperature in a desiccator, the crucibles were weighed (W1). Subsequently, the crucibles were placed in a muffle furnace at 550 °C for 3 h, then cooled in the desiccator and reweighed as W2 (i.e., acid–insoluble ash). The amount of acid–insoluble lignin (also termed Klasen lignin) was determined as W1–W2.

Monosaccharides analyzes were performed by HPAEC-PAD analysis using an ICS-3000 system consisting of a gradient pump (model DP-1), an electrochemical detector/chromatography module (model DC-1) and autosampler ( Dionex Corp., Sunnyvale, CA). Separation was achieved using a CarboPacTM PA20 (3 mm × 150 mm) analytical column following that described by Arnoux and Meyer (2008). Roughly, it is considered that arabinose, galactose, galacturonic acid and rhamnose are specific to pectins, and glucose belongs to the cellulose moiety (Crônier et al., 2005; Vignon and García-Jaldon, 1996). The concentration of polymeric sugars was calculated from the concentration of the corresponding monomeric sugars, using an anhydrous correction of 0.88 for C-5 sugars and a correction of 0.9 for C-6 sugar (Sluiter et al., 2011).
2.4. Microscopic and histochemical observations

Fresh transverse sections were cut from the bottom regions of hemp stems using a stereo microscope (Wild Heerbrugg M8, Wild Leitz, Switzerland). Histochemical staining was performed on transverse hemp stem sections on glass slides followed by adding one drop of 50% (v/v) glycerol in water. Cover slips were placed on the stained sections, mounted in glycerol and examined immediately using a Leica DMLB light microscope (LM) with digital images recorded using an Infinity X-32 camera (DeltaPix Denmark). The following histochemical reactions were performed (Thygesen et al., 2005):

2.4.1. Lignin-hydroxycinnamyl aldehydes (Wiesner reaction)

The stem sections were stained with two drops of 10 g/L phloroglucinol in ethanol followed by the addition of one drop of 35% (v/v) HCl.

2.4.2. Syringyl lignin (Mäule reaction)

The stem sections were stained with one drop of 1% (w/v) aqueous KMnO₄ for 5 min. followed by three washes in water. The stem sections were then immersed in 3% (v/v) HCl for 1 min, washed with distilled water and immersed in 29% (v/v) NH₃ for 1 min, followed by washing with distilled water.

Fig. 1. Precipitation and daily average temperature in the trial location during the period of growth of the hemp plants and field retting (Meteo France, Weather station Mouroux no. 77320002).

Fig. 2. Flow diagram showing sample preparation in this study.
2.4.3. Pectin

The stem sections were stained with 0.02% (w/v) aqueous ruthenium red (JMC Specialty Products).

Due to the temporary nature of the staining reactions, all observations including digital image recording were performed within 10–15 mins of staining. A number of morphological features were measured using Image-Pro Plus (Media Cybernetics Inc., USA), including thickness and area of epidermis, primary fiber layer (PFL), secondary fiber layer (SFL), cambium, fiber cell walls and lumen. Thus the PF- (i.e., ratio of the PFL area to the bast area) and SF fractions (i.e., ratio of the SFL area to the bast area) could be determined.

Morphological features at hemp stem level were determined as the average of 4 measurements from 10 transverse sections from 3 bundles of each sample, which in total represented 120 measurements. At the single fiber level, an average of 4 measurements from 50 cells from 3 fiber bundles of each sample of bast fibers were made representing in total 600 measurements.

2.5. Tensile strength testing of fiber bundles

The bast fiber strips (80 mm long × 1 mm wide) were peeled manually from the hemp stem followed by minor modification of the strips with a razor blade to obtain constant width along the entire length of the bast fiber strips using a light microscope. The main part of the epidermis of each strip was removed using a razor blade and the center part of the strip, with length 60 mm, was excised for tensile testing. The other two pieces (each 10 mm in length) were embedded in epoxy resin and the cross-sectional area determined by optical microscopy combined with image analysis. The average cross-sectional area was applied in the further calculations. A sketch and a micrograph of a typical transverse section of the bast fibers are shown in Fig. 3A. Tensile tests were carried out on 20 specimens at each treatment level.

Test specimens were made by gluing tabs on each fiber tip with epoxy resin (DP 100) with a custom-made holder (Fig. 3B). Considering the short length of single fibers in test specimens, a gauge

---

Fig. 3. The micrograph showing a transverse section of a bast fiber strip (A) and the setup used for sample preparation for tensile strength testing (B).

Fig. 4. Schematic diagram of a transverse section of hemp stem showing the organization and morphology of a bast strip and single fiber (e.g., primary- and secondary fibers) in the bast layer.
length of 10 mm was used. Bast fiber strips were first placed on the surface of the bottom tabs, aligned along the centerline and fixed at two adhesive points, keeping the test pieces straight. Then, a drop of epoxy resin was applied to the center of the bottom tabs and the upper tab placed on top. Finally a cover-plate was used to clamp the tabs using screw nuts. After curing at 20 °C for 24 h, the fixed samples were removed from the holder.

All samples were tensile tested using an Instron Testing Machine 2710-203 equipped with a 1000 N load cell, at a tensile speed of 0.5 mm/min at 25 °C and 50% humidity. The ultimate tensile strength (UTS) of the bast strips was defined as the ratio of failure load (N) and the cross-sectional area of the bast strips including the area of fiber cell walls and lumen.

2.6. Statistical analysis

Analysis of variance (ANOVA) for the two growth stages was performed on each measurement. For each morphological characteristic, chemical composition and mechanical properties, maturation effect was tested at a significance level of 5% (Minitab 16). For the mechanical properties of retted fibers, ANOVA was performed independently on the measurements of early- and late harvest samples and the field retting duration effect was tested. Differences between each retting time were evaluated using the Turkey multiple comparison test with a level of significance at 5%.

3. Results and discussion

3.1. Hemp stem morphology

The original hemp stems were 1.5–2.5 m tall and 5–15 mm in diameter. The stems contained 30–40% w/w bast fibers and were organized in layers from the stem pith toward the surface by 1–5 mm xylem, 10–50 μm cambium, 100–300 μm cortex, 20–100 μm epidermis and 2–5 μm cuticle at the macroscopic level. At the microscopic level, the bast fibers included primary- and secondary fibers. The definition of stem components was in accordance with that reported by Garcia-Jaldon et al. (1998) and Schäfer and Honermeier (2006) and shown schematically in Fig. 4. A large variation in the morphological features of hemp bast fibers from bottom section to top section was observed. Only the bottom section was therefore investigated in this study.

3.2. Microscopic observations of hemp bast fibers from different stem sections

A microscopic image of a transverse section illustrating the cellular structure of developing hemp stems is shown in Fig. 5 and morphological characteristics at stem- and single fiber levels shown in Tables 1 and 2, respectively. Great variations in the morphology of hemp stems were apparent with maturity providing interesting information on the variability at both bast fiber- and individual fiber levels. However, the same overall organization of layers from the innermost xylem toward the surface consisting of cambium, secondary- and primary fibers, epidermis and cuticle were observed for both developmental stages (Fig. 5A and B).

At the bast fiber level, there was a significant increase in thickness of secondary fiber layer (SFL) from 10 to 54 μm from early- to late harvest, but major reductions in the thickness of epidermis and primary fiber layer (PFL) were observed (Table 1). As a result, the primary fiber (PF) fraction decreased from 49 to 45% (non-significant at P = 0.05) and secondary fiber (SF) fraction increased significantly from 2 to 10%.

Hemp is characterized by the presence of secondary fibers which are shorter and thinner than primary fibers (Mishra 2009; Sankari 2000a). Amaducci (2008) reported the secondary fibers as primarily present in the bottom of plant stems. Besides the effect of fiber location inside hemp stems, Schäfer and Honermeier (2006) reported that the proportion of secondary fibers was affected by weather (i.e., dry conditions) and concluded that harsh weather results in more secondary fibers. According to our microscopy observations, the proportion of secondary fibers increased with period of growth. At the beginning of flowering, only a few secondary fibers surrounded by many parenchyma cells existed in the inner part of the bast fiber

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stem diameter (mm)</th>
<th>Bast content (%)</th>
<th>Xylem content (%)</th>
<th>EL thickness (μm)</th>
<th>PFL thickness (μm)</th>
<th>SFL thickness (μm)</th>
<th>PF fraction&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>SF fraction&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early harvest</td>
<td>7.2 ± 0.8</td>
<td>44 ± 1</td>
<td>56 ± 1</td>
<td>89 ± 23</td>
<td>184 ± 47</td>
<td>10 ± 3</td>
<td>49 ± 3</td>
<td>2 ± 3</td>
</tr>
<tr>
<td>Late harvest</td>
<td>8.6 ± 0.9</td>
<td>37 ± 1</td>
<td>63 ± 1</td>
<td>52 ± 21</td>
<td>119 ± 10</td>
<td>54 ± 4</td>
<td>45 ± 5</td>
<td>10 ± 6</td>
</tr>
</tbody>
</table>

<sup>a</sup>F value for harvest time effect at P<0.05 (*), P<0.01 (**), and P<0.001 (**).<br/>

<sup>b</sup>PF- and SF fractions were defined as the ratio of transverse area of primary- and secondary fiber in the fiber strip to the sum of the transverse area of epidermis layer, primary fiber layer, secondary fiber layer and cambium layer, respectively.
layer showing a low abundance (Fig. 5A). In contrast, a distinct and thick secondary fiber layer arranged into separated fiber bundles was observed at seed maturity (Fig. 5B).

The results are consistent with previous studies (Mediavilla et al., 2001; Schäfer and Honermeier, 2006), which show that the development of secondary fibers starts at the early flowering stage and more secondary fibers are formed during the latter part of seed ripening. In addition, it is interesting that the bast content was reduced from early- (44%) to late harvest (37%). In addition, the plant density of 250 per m² at emergence decreased to 140 per m² at harvest. This indicates that self-thinning occurred. This reduction in plant density may be ascribed to inter-plant competition, which is found to cause density-induced mortality resulting in a reduction of bast content in the stem (Van der Werf et al., 1995a,b).

At the single fiber level, morphological information with respect to primary- and secondary fiber were observed (Fig. 5). Results were consistent with previous studies on hemp bast fibers, where variations in fiber diameter have been reported (Crônier et al., 2005; Ouajai and Shanks, 2005; Placet et al., 2012; Schäfer and Honermeier, 2006; Wang et al., 2007). In Beckermann and Pickering (2008), the hemp fiber diameter was on average between 20 and 33 μm. However, since the fiber cells have different shapes (e.g., round, oval, polygonal), precise measurements of their diameter are almost impossible (Fig. 5). Therefore, in our study, the area of fiber cells was determined (Table 2). A reduction in the thickness of both primary- and secondary fiber cell walls with increasing maturity was found, while the reduction in the thickness of primary fiber cell walls was statistically significant (P < 0.05). However, the cell area of primary fibers was found to increase from 1416 to 1500 μm². Furthermore, the slight decrease in the proportion of lumen (i.e., porosity) from 8.2 to 7.9% indicated that the investigated fibers were already mature at the beginning of flowering. This is in agreement with the results reported by Amaducci et al. (2008) who found that hemp fibers from bottom sections of stems reached the highest maturity at the beginning of flowering.

It is interesting that the reduction in primary fiber- and secondary fiber cell wall thickness and area coincided with a reduction in bast content, while the proportion of secondary fibers showed a significant increase (Table 1). Presumably, inter-plant competition (indicated by a high decrease in plant density from 140 per m² to 40 per m² from early to late harvest), and the relatively dry and hot weather conditions between early and late harvest period (Fig. 1) are likely responsible for this results.

The foregoing description on variations in morphological characteristics for the two development stages shows that the morphological features of hemp fibers are strongly influenced by growth stages. It appears characterized by the non-terminating growth of fibers while new secondary fibers are continually generated from separated single cells at earlier growth stages (i.e., at the beginning of flowering). Secondary fibers are then organized into bundles which produced a distinct layer in the mature stem in contrast to that observed for primary fibers, which have been arranged into a layer since early harvest. In addition, the proportion of parenchyma cells among fiber bundles continued to increase (Fig. 5A and B). These changes were assumed associated with seed formation between early- and late harvest.

### 3.3. Chemical analysis and histochemical staining of transverse sections of hemp stems

The chemical components of bast fibers from early- and late harvest are presented in Table 3. Notably, the lignin content increased significantly from 3.4 to 4.8% over the growth period from the beginning of flowering to seed maturity. In contrast, a highly significant (P < 0.01) reduction in cellulose content (indicated by glucose content) and decrease in pectin deposition (indicated by galacturonic acid content) were noted from the beginning of flowering until seed maturity. The reduction in cellulose content was consistent with the morphological characteristics of bast fibers revealed by the microscopy investigations, which showed a decrease in cell wall thickness of both primary- and secondary fibers with maturity.

Histochemical analyzes were performed for visualizing the spatial micro-distribution of lignin and pectin in the wall structure of hemp cells. Pectins are non-cellulosic acidic polysaccharides that are found primarily in the compound middle lamellae (CML) of plant cell walls (i.e., between fibers, parenchyma etc.) with much lesser amounts in secondary walls. Ruthenium red staining has frequently been used for localizing pectin in plant/wood tissues and stains acidic pectins red/pink (Hou et al., 1999; Waller et al., 2004). The staining of transverse sections of hemp stem showed red/pink staining and presence of pectins in the CML region between all cell types (Fig. 6A and B) irrespective of the growth stage. In addition, greatest staining was shown in cell corner middle lamellae (ML) of hemp fibers indicating presence of pectins at high concentration. This suggests that fiber separation could be achieved by pre-treating pectin either by its partial removal or structural change in the ML region during retting. However, a difference in the staining intensity between the two developmental stages (Fig. 6A vs B), where early harvested hemp stems stained more strongly than late harvested stems were consistent with the chemical analysis during ripening of the hemp plants (Table 3).

The spatial micro-distribution of lignin within hemp cell walls during the two growth stages was determined using the Wiesner- and Mäule reactions (Fig. 6C–F). Phloroglucinol in the Wiesner reagent reacts with lignin guaiacyl units producing a deep red color while the Mäule reaction stains lignin brown/orange due to the reaction with syringyl units in lignin.

Although the xylem cell walls stained an intense red color with Wiesner reagent irrespective of the growth stage, differences in staining were observed in the bast region of the stem with respect to cell type and their growth phase (Fig. 6C and D). Similar observations were obtained with the Mäule reagent where a positive reaction for syringyl lignin was detected in the cell walls (Fig. 6E and
Table 3
Anhydrous monosaccharides and Klason lignin content of investigated hemp fibers (standard deviations of 3 replicates in parentheses)

<table>
<thead>
<tr>
<th>Harvest time</th>
<th>Retting time</th>
<th>Amount (g/100 g dry matter)</th>
<th>Glu</th>
<th>GaIA</th>
<th>Gal</th>
<th>Xyl</th>
<th>Man</th>
<th>Ara</th>
<th>Rha</th>
<th>Klason lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early harvest</td>
<td>0</td>
<td>66.0 (1.5) 6.9 (0.4) 2.0 (0.2) 1.0 (0.0) 2.8 (0.3) 1.0 (0.1) 1.0 (0.1) 3.4 (0.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late harvest</td>
<td>0</td>
<td>61.3 (0.4) 6.3 (0.3) 2.1 (0.1) 1.1 (0.2) 3.0 (0.3) 1.0 (0.1) 1.1 (0.0) 4.8 (0.5)</td>
<td>P&lt;0.01</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Early harvest</td>
<td>50</td>
<td>71.3 (0.5) 3.1 (0.3) 2.4 (0.1) 1.1 (0.1) 3.8 (0.4) 0.4 (0.0) 0.8 (0.0) 4.3 (0.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late harvest</td>
<td>7</td>
<td>63.9 (1.0) 5.4 (0.6) 2.0 (0.2) 1.1 (0.2) 4.0 (0.4) 0.7 (0.2) 1.0 (0.0) 5.3 (0.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early harvest</td>
<td>70</td>
<td>65.1 (2.6) 4.1 (0.4) 2.0 (0.0) 1.5 (0.0) 4.9 (0.3) 0.6 (0.1) 0.8 (0.0) 7.4 (1.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late harvest</td>
<td>14</td>
<td>70.2 (0.5) 3.1 (0.4) 2.4 (0.2) 0.8 (0.2) 3.4 (0.1) 0.4 (0.0) 0.8 (0.0) 6.1 (0.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early harvest</td>
<td>21</td>
<td>66.9 (1.2) 3.6 (0.4) 2.0 (0.0) 1.0 (0.2) 3.9 (0.2) 0.6 (0.1) 0.8 (0.0) 8.1 (0.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


P value: the effect of harvest time on chemical composition of different samples, ns: non significant.

F). Although there was a positive reaction in both primary- and secondary fibers, both reagents stained the CML strongly and also the outermost layers of the secondary walls compared to inner regions which showed very pale or no staining (Fig. 6). This indicated that lignin is predominantly concentrated in CML regions of bast fibers with much lesser amounts in secondary walls. Results are consistent with the plant’s classification as an Angiosperm, which are known to contain both guaiacyl and syringyl lignin units.

In addition, the bast fibers from the two growth stages stained differently according to plant growth stage. Despite positive
reactions for both primary- and secondary fibers as seen in Fig. 6C–F, the outermost primary fibers (close to epidermis) in stems at seed maturity stained with greater intensities indicating a higher lignin content in the most matured fibers presumably due to the deposition of lignin during the seed maturity stage. Results suggest that lignin deposition continued from the beginning of flowering to seed maturity thus correlating with the chemical composition analyzes.

Variations in relative carbohydrate and lignin components during field retting for both early- and late harvests were investigated. The relative and normalized content increase in lignin, pectin and cellulose are shown in Fig. 7. The cellulose content increased more than 10% during retting for both early- and late harvest, but decreased at later stages of the retting process. The pectin content decreased slightly at the beginning of retting and thereafter remained stable. In contrast, the lignin content increased steadily during the whole retting period for both early- and late harvested hemp. Results suggest that the cellulose content is highest at the early part of field retting whereas the lignin content becomes concentrated during the retting period. The results correlate with the normalized content increase (Fig. 7A2 and B2) and that cellulose, pectin and lignin are degraded at different rates. Pectin is removed at the highest rate, followed by cellulose and then lignin.

The changes in polymer composition can be explained by the action of microorganisms, which degrade and dissolve the cellular tissues and pectins surrounding the bast-fiber bundles (Fu et al., 2011), so facilitating further separation of the fibers from the stem (Di Candilo et al., 2010). When hemp stems start to degrade, fungal colonies appear as dark flecks on the surface of the bark and continue to develop until the surface turns to a steel-grey color (Jankauskienė and Gruzdevienė, 2013). At the initial stage of attack, growth of microorganisms is vigorous but as retting proceeds, the growth rate decreases (Donaghy et al., 1990). Therefore at the early stage of retting, pectins are degraded very rapidly (Fig. 7A2 and B2) with the rate of degradation decreasing as retting continues. In contrast, the rate of cellulose degradation increased gradually with period of retting. Presumably, as pectin is degraded and removed, the accessibility of cellulose for the microorganisms increases, and therefore a decrease in cellulose content is shown during the later stages of retting.

3.4. Mechanical properties of non-retted and field retted hemp fibers

The mean values for mechanical properties of the bast strips isolated from non-retted and field retted hemp stems are presented in Table 4. The mean values for ultimate tensile strength (UTS), elongation and stiffness were 683–954 MPa, 4.5–6.2%, and 27.5–34.9 GPa, respectively. Similar values for mechanical properties of hemp fibers are given in the literature (Table 5) with 489–899 MPa, 2.1–4.4%, and 27.6–66 GPa, respectively. Generally, the data verify that the method in this study for tensile testing gives reasonable values for UTS and stiffness, while the value for elongation is a little higher (Table 5).

Results indicate that the mechanical performance of hemp fibers was strongly influenced by harvest time. While fibers
Mechanical performance of hemp fibers is shown in Table 4. Industry because of its low cost. The effect of field retting duration fiber damage. Field retting (or dew retting) is still widely used in tion) of fibers from stems and is essential for the reduction of inferior fiber quality.

The variation of the mechanical properties of hemp fibers with harvest time can be explained by results from microscopy observations and chemical analyzes. A noticeable decrease in bast content and increase in the proportion of the secondary fibers (Table 1) during seed maturation were observed in the present study. The data obtained are in agreement with previously published reports where hemp fiber quality was found to be reduced with decreasing bast content, which may have resulted from self-thinning (Van der Werf et al., 1995). In addition, Mediavilla et al. (2001) reported a reduction in fiber yield and quality after the flowering stage due to the formation of secondary fibers. Furthermore, the reduction of mechanical properties with maturity may also be closely related to the statistically significant decrease in cellulose deposition (Table 3) since the importance of cellulose on the tensile properties of fibers retted for 7 days in the field with non-retted samples and the reduction in elongation at break was not statistically significant until the period of retting reached 70 days. However, no significant decrease in stiffness was observed. These results may reflect the accelerated cellulose degradation during the latter part of field retting as indicated by the negative slope of the line for cellulose content in Fig. 7A and B.

Results for the late harvest stems were similar to those from the early harvest although some interesting differences were noted. In particular, no statistical significant reduction in mechanical performance of fibers was observed compared with non-retted samples, although both UTS and stiffness decreased with the extended period of field retting. In addition, there was a slight increase in mechanical properties of fibers retted for 7 days in the field with respect to UTS and stiffness and then a notable reduction of both parameters with increasing retting time. This may be explained from early harvest hemp stems exhibited significantly higher mechanical properties with an UTS and elongation at break and stiffness of 954 MPa, 6.2% and 34.9 GPa, respectively, those from the late harvest possessed 812 MPa, 4.6% and 31.1 GPa, respectively.

### Table 5

<table>
<thead>
<tr>
<th>Hemp variety</th>
<th>Harvest year</th>
<th>Tensile strength at break (MPa)</th>
<th>Stiffness (GPa)</th>
<th>Elongation at break (%)</th>
<th>Gauge length (mm)</th>
<th>Tensile speed (mm/min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fedora 17</td>
<td>2011</td>
<td>889 ± 472</td>
<td>35.5 ± 17.3</td>
<td>2.6 ± 2.2</td>
<td>10</td>
<td>1</td>
<td>Marrot et al. (2013)</td>
</tr>
<tr>
<td>Felina 32</td>
<td>2009</td>
<td>699 ± 450</td>
<td>31.2 ± 19.7</td>
<td>3.3 ± 1.6</td>
<td>10</td>
<td>1</td>
<td>Marrot et al. (2013)</td>
</tr>
<tr>
<td>Fedora 17</td>
<td>2007</td>
<td>489 ± 233</td>
<td>33.8 ± 12.2</td>
<td>2.5 ± 1.3</td>
<td>10</td>
<td>1</td>
<td>Marrot et al. (2013)</td>
</tr>
<tr>
<td>Unknown 2002</td>
<td>857 ± 260</td>
<td>58</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>Pickering et al. (2007)</td>
</tr>
<tr>
<td>Unknown</td>
<td>/</td>
<td>1061 ± 253</td>
<td>27.6 ± 7.5</td>
<td>2.1 ± 0.7</td>
<td>8</td>
<td>0.12</td>
<td>Placet et al. (2012)</td>
</tr>
<tr>
<td>USO-31</td>
<td>1995</td>
<td>830 (661, 1125)</td>
<td>/</td>
<td>4.3 (3.2, 6.5)</td>
<td>20</td>
<td>20</td>
<td>Sankari (2000)</td>
</tr>
<tr>
<td>USO-31</td>
<td>1996</td>
<td>647 (478, 942)</td>
<td>/</td>
<td>4.4 (2.6, 5.4)</td>
<td>20</td>
<td>20</td>
<td>Sankari (2000)</td>
</tr>
</tbody>
</table>

* Values denote median (25th percentile, 75th percentile).
by the significant increase in cellulose content in retted samples because of the microbial removal of pectins during field retting (see Fig. 7B1–B2). However, in later stages, cellulose degradation was accelerated after most of the non-cellulosic tissues were removed and accessibility of cellulose to the microorganisms was increased. Together, the effect of field retting appears largely dependent on retting time. When retting duration was shorter, the mechanical properties of fibers with respect to UTS and stiffness may increase due to a continuous concentration of the cellulose content. However, the mechanical properties varied inversely with extended retting time probably due to accelerated cellulose degradation as a result of increasing accessibility of microorganisms to cellulose.

It should be noted that hemp fibers are categorized as “natural cellulosic fibers”, whose properties can be influenced by many parameters during their growth and development (Duval et al., 2011). Furthermore, bast fiber strips can be regarded as fiber-reinforced composites, whose properties depend not only on those of the fibers themselves but also on the degree to which an applied load is transmitted to the fibers by the matrix phase under stress (Callister, 1994). The mechanical performances of the bast fiber strips therefore depend on both the mechanical properties of the individual fibers and coherence between fibers. Thus, the removal of non-cellulosic polymers during field retting results in lower coherence between fibers and thereby in lower mechanical properties.

4. Conclusions

The development of hemp fibers as reinforcement agents for composites and the requirement of high mechanical performance of cellulosic fiber reinforced materials require optimal fibers and a corresponding economical and accurate method for fiber extraction. Thus, an improved understanding of the properties of non-retted fibers and effects of field retting on the chemical composition and mechanical properties of fibers is of major importance.

Fibers harvested at seed maturity are significantly lignified and thus will be difficult to be extracted from hemp stems compared with fibers from the beginning of flowering. Furthermore, the important reduction in hemp bast mechanical properties with plant maturity may be attributed to the combined effect of the noticeable decrease in cellulose deposition, and the formation and increase in proportion of secondary fibers that caused deterioration of primary fiber quality regarding morphological and chemical characteristics. Highly lignified fibers are not desirable and favorable for retting. Considering the mechanical performance of hemp bast fibers, hemp harvesting at the beginning of flowering is therefore recommended for use in strong composites.

In this study, the reduction in fiber quality caused by a long period of field retting was confirmed and may be related to a continuous increase in cellulose degradation, while no noticeable change in fiber quality was observed for short-periods of field retting. Consequently, traditional field retting may not be the optimal pretreatment for strong fibers and it is suggested that short-period field retting may be adopted to extract fibers more efficiently and accurately combined with other methods including the use of targeted enzymes, selected microbes or chemicals.

Acknowledgements

The authors are grateful to the Danish Council for Independent Research supporting the CelFilMat project (IP No. 12-127446: “High quality cellulosic fibers for strong biocomposite materials”). The financial support of China Scholarship Council (CSC, no. 201304910245) for Ming Liu’s Ph.D. project is acknowledged. Tomas Fernqvist, Annette Eva Jensen and Jonas Kreutzfeldt Heinige are thanked for technical support.

References


