Flax treatment with strategic enzyme combinations: effect on fiber fineness and mechanical properties of composites

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Abstract

The application of natural fibers as reinforcement material in composites can make an important contribution towards a more sustainable bio-based economy. To use these natural fibers, they need to be released from the stem. Traditionally, this is realized by dew retting. However, there are some disadvantages to this retting procedure. Due to changing weather conditions, it is quite difficult to guarantee a good and consistent fiber quality. Replacing the traditional retting process by a biocatalytic process may resolve these issues through application of a combination of pectinase and hemicellulase activities. In this context, several strategic pectinase and hemicellulase activities were blended. Flax fiber fineness and mechanical properties of resulting unidirectional fiber-epoxy composites were studied after treatment of flax stems with these strategic enzyme combinations. Characterization of fiber fineness showed a higher percentage of fiber widths smaller than 100 μm after combined treatment with two enzymes (44.7 %) and the percentage was even higher after some combinations with three enzymes (51.2 %). Assessment of mechanical properties of composites showed no significant differences in tensile or transverse properties for combined enzymatic treatments. Fiber properties were back-calculated from unidirectional flax fiber-epoxy composites and a Young's modulus of 88 GPa and a tensile strength of 865 MPa were observed. Enzymatic extraction resulted in the achievement of similar results as dew retting concerning mechanical composite performance and fiber fineness.

Keywords: natural fibers, composites, mechanical properties, enzyme combinations

1. Introduction

The replacement of glass fibers by natural fibers in composite materials can make an important contribution to the next generation of composites in a more sustainable and bio-based future. Compared to glass fibers, natural fibers exhibit several advantages: lower density, high specific mechanical properties, reduced cost, high availability, biodegradability and eco-friendly nature [1, 2, 11, 3–10]. Next to wood and cotton fibers, flax, jute, hemp and sisal are frequently used as reinforcement material for high value applications [1, 4]. Biofibers can differ in their mechanical properties, being mainly influenced by the ratio of cell wall thickness to fiber diameter, morphology of the fiber, cellulose crystallinity, polysaccharide composition of the cell walls and the microfibrillar angle of the cellulose fibrils [4].

In Europe, flax and hemp are the most frequently produced plant fibers [4]. Due to the high content of crystalline cellulose (55 - 75 %) and the low microfibrillar angle of 10°, flax is one of the most interesting and promising natural fibers [4, 12–16]. Flax fibers are most frequently used for high-quality textiles but during the last decades, the importance of flax fibers as reinforcement in high-quality composites keeps increasing [17].

Traditionally, flax fibers are extracted by a dew retting process in the field, followed by a mechanical extraction procedure. Dew retting however requires regions with appropriate moisture and temperature conditions, necessary for good microbial growth [18, 19]. This dependence on weather, climate and region and the long duration of the process, results in flax with a high variety in chemical, morphological as well as mechanical properties. Furthermore, the additional mechanical treatment to extract fibers from the woody stem increases fiber damage and impairs the quality of the fibers. For the production of high value biocomposites, high-quality fibers with a consistent performance are needed.

The substitution of dew retting by enzymatic retting can offer a solution for the inconsistency in fiber quality and fiber damage by the controllability of the process and specificity of the enzymes. In addition, the general applicability of natural fibers in composite materials can be improved by rethinking the fiber extraction process with minimized variability and fiber damage [5]. Loosening and separation of the fibers from the woody stem can be accomplished by the specific degradation of the surrounding polymeric network of the fiber [20]. The mechanical post-treatment afterwards will be less severe and hence will induce less fiber damage resulting in a higher fiber yield.

To achieve high-quality fibers, insights are needed which enzyme activities contribute most towards an easy release of the fibers from the stem. Moreover, combining the key enzyme activities may further enhance resulting fiber fineness and composite properties forming the major objective of this research. Previous work within our research group and others demonstrated that the individual enzyme activities polygalacturonase, pectate lyase and xylanase are the most appropriate in view of the biochemical and mechanical fiber properties as well as composite quality [5, 20–26]. In De Prez et al. (2019), fibers were manually extracted to fully comprehend the effect of the single enzymatic action and exclude property changes by mechanical treatments. Through this, fiber damage could be minimized and very promising fiber properties were obtained, i.e. technical fibers with a back-calculated Young's modulus of 84 GPa and strength up to 800 MPa [25]. In literature on the other hand, stiffness values of 27 to 80 GPa were reported [10], probably including properties of technical as well as elementary fibers (which normally possess a higher stiffness). Bourmaud et al. [4] reported Young's modulus results of flax fibers between 37 and 75 GPa [12, 27–30]. The obtained mechanical properties are hence very promising for the production of high-quality unidirectional composites.

Based on the previous insights, strategic enzyme mixtures were studied within this research to investigate if further reduction in fiber fineness and further improvement of mechanical composite performance is feasible. Hereby, this research was focused on flax.

Some research groups have tested some combinations of enzymes for their effect on natural fibers previously. Research of George et al. [31] showed that fibers treated with the combination of xylanase + cellulase were better suited for impregnation in polypropylene composites due to the better water resistance and higher thermal stability of the composite. The composites however exhibited no improvements in mechanical properties compared with fiber treatments with individual enzymes [31]. Stuart et al. [32] tested a commercial enzyme solution, 'Pectinex AR', which exhibited high xylanase, polygalacturonase and cellulase activities. However, treatments were also performed on already extracted fibers and composites were non-woven laminates with randomly oriented fibers as reinforcement, while this research aims at production of unidirectional composites based on natural fibers being enzymatically extracted instead of applying the traditional dew retting process. A stepwise combination of different enzyme activities based on results of individual enzyme performance to produce high-quality unidirectional composites is however of utmost importance.

Endopolygalacturonase (EPG) was studied by Akin et al. [22] separately and in combination with other enzyme activities, i.e. with pectin lyase, pectin methylesterase, xylanase and endoglucanase. Enzymatic treatments on flax stems were evaluated with Fried Test scores, fineness and fiber strength determination and addition of supplemental enzymes to the EPG solution produced fibers with similar properties than after EPG treatment alone [22]. Foulk et al. [33] investigated the effect of combinations of several commercial enzymes from Inotex. The combinations were evaluated with a Fried Test but did not show any clear improvements either [33]. The Fried Test however is an operator dependent test method.

Characterization of chemical fiber properties along with mechanical properties are important factors to consider in the evaluation process of enzymatic treatments. Furthermore, as elucidated in De Prez et al. [5], mechanical properties of fibers cannot be extrapolated to mechanical properties of the composite. Determination of mechanical properties of composites based on enzymatically extracted fibers is therefore indispensable to gain insight in the quality of the intended product. Enzymatic treatments should be evaluated with standardized protocols for determination of chemical composition of the fiber, extraction efficiency, fineness of the fiber and mechanical properties of the biocomposites. The effect of strategic enzyme combinations on fiber fineness and mechanical properties of unidirectional flax fiber-epoxy composites was therefore assessed within this paper. Fineness of the extracted fibers illustrates the influence of enzymatic action on the loosening and separation of fibers from the woody core of the flax stem. In view of applying the extracted flax fibers as reinforcement for high-quality unidirectional composite materials as alternative for glass fiber reinforced composites, the mechanical properties of the flax fiber-epoxy composites should also be addressed.

2. Materials and methods

2.1 Materials

As starting material for enzymatic treatments, green flax harvested by Verhalle (Belgium) in 2015 was used. The flax company Verhalle provided dew retted flax stems from the same cultivar (Amina). Fibers were extracted from green flax and dew retted flax through a manual procedure, resulting in green fibers (GR) and manually extracted dew retted fibers (DRm). In contrast, dew retted flax mechanically processed by scutching, i.e. scutched dew retted fibers (DRs), was provided by Vanacker Rumbeke and harvested in France in 2017. Furthermore, FlaxTape (FT) from Lineo, having a density of 200 g/m², was included as well

for comparison. FT is a commercially available product whereby the flax fibers also underwent a hackling procedure. The cultivar is however unknown.

Enzymatic treatments were performed with Scourzyme L (Sc), NS59049 (NS) and Pulpzyme (Pz) from Novozymes (Switzerland). Rohapect MPE (MPE) was provided by AB Enzymes (Germany). Polygalacturonase from *Aspergillus niger* (PAn) and xylanase from *Thermomyces lanuginosus* (XTI) were purchased from Sigma-Aldrich. Characterization of enzyme activities was studied and described in De Prez et al. [20].

2.2 Enzymatic treatment

Strategic enzyme combinations were made with several pectinases and hemicellulases: Scourzyme (a pectate lyase), NS59049 (a pectin lyase), polygalacturonase from *A. niger* (PAn), Rohapect MPE (a pectin methylesterase), Pulpzyme (endoxylanase) and xylanase from *T. lanuginosus* (XTI). Enzymatic treatments were performed by incubating 50 g green flax stems at 40 °C during 24 h in a 1 l enzyme formulation of pH 6.5, containing 25 mM EDTA and 0.30 v/v % enzyme (in case of PAn 0.60 v/v %) as shown in Figure 1.

Figure 1. Overview of manufacturing process

Afterwards, flax stems were washed twice, dried (24 h at 105 °C) and fibers were manually extracted as described in De Prez et al. [34]. Additionally, two reference treatments were performed on green flax stems, i.e. water treatment (with tap water at 40 °C during 24 h) and EDTA treatment (with 25 mM EDTA at 40 °C and pH 6.5 during 24 h). Each enzymatic treatment was performed twice.

2.3 Fineness measurement

Fiber fineness was characterized by using an automated image analysis method as described in Hendrickx et al. [35] and De Prez et al. [34], which measures fiber diameters through scanning 30 to 40 fibers with a flatbed scanner at a resolution of 2400 dpi. As a result, distribution curves of the fiber diameters are produced.

2.4 Composite production

Production of unidirectional flax fiber-epoxy composites was performed according to the Impregnated Fiber Bundle Test methodology (IFBT), based on ISO 10618. Fiber bundles immersed with resin were placed in a vacuum bag between mould and counter mould. In the manual press, pressure was equally distributed on the surface by placing a Teflon film and a layer of breather on top of the mould and pressure was retained for 1 hour at 70 °C, followed by a post-curing step during 1 hour at 150 °C. For each batch of enzymatic treatment, extracted fibers were used to produce at least 4 composite specimens in case of tensile testing or 6 to 7 composite specimens in case of bending tests. A more detailed description of the protocol is discussed in De Prez et al. [25].

2.5 Tensile testing

An Instron 5567 was used for testing longitudinal tensile properties of the composites, with a load cell of 30 kN and a crosshead displacement rate of 2 mm/min, according to ASTM D3039 [36]. The gauge length was 150 mm and strain was measured with a 50 mm extensometer on the middle of the sample. In addition to tensile strength of the composite, two tensile moduli were determined: E1, the stiffness between 0.0 and 0.1 % strain and E2, the stiffness between 0.3 and 0.5 % strain, since in case of flax fiber composites a typical decrease in stiffness can be observed around 0.2 % strain [37].

2.6 Three-point bending testing

Transverse bending strengths of the composite samples were measured by three-point bending on an Instron 5567 with a load cell of 1 kN, according to ASTM D7264 [38]. The crosshead displacement rate

was 0.5 mm/min. The diameter of the loading nose was 6 mm while the diameter of the support rolls was 4 mm, with a span length of 16 mm.

2.7 Fracture surface analysis with Scanning Electron Microscopy

The fracture surface of composite samples subjected to three-point bending tests was analyzed with a Scanning Electron Microscope (SEM, Philips SEM XL30FEG, Netherlands). A thin gold/palladium film was sputtered on the samples as a conductive coating and samples were kept under vacuum until imaging by SEM using a beam voltage of 10 kV.

2.8 Statistical analysis

Statistical analysis was performed by running one-way Analysis of variance (ANOVA) in SPSS with a confidence level of 95 % and means comparison with a Tukey post hoc test.

3. Results and discussion

3.1 Enzyme combinations

This study investigates the effect of flax treatment with enzyme combinations on fiber fineness and mechanical properties of fiber-epoxy composites. Enzyme combinations were created by combining six enzyme activities: pectate lyase (Sc), pectin lyase (NS), pectin methylesterase (MPE), polygalacturonase (PAn), endoxylanase (Pz) and xylanase (XTI). These enzymes have been extensively studied towards their effect on the ease of fiber extraction and chemical composition of the manually extracted fiber [20]. Pectate lyase, polygalacturonase and xylanase came forward as most promising enzymes for their retting efficiency [20]. Furthermore, single enzymatic treatments with these enzymes have been characterized

for their effect on fiber fineness and mechanical properties of composites [25]. Fiber properties were back-calculated from composite properties. Fibers with tensile moduli from 60 to more than 80 GPa and a tensile strength from 600 to 800 MPa were observed [25]. Based on these insights, different enzymes were strategically combined to study possible synergism and improved retting efficiency. An overview of the strategic combinations is schematically represented in Figure 2.

Figure 2. Overview of strategic enzyme combinations with (A) polygalacturonase (PAn), (B) xylanase (XTI), (C) polygalacturonase + pectin methylesterase (PAn+MPE) and (D) two additional combinations with three enzymes

Polygalacturonase is one of the most important enzymes necessary to perform retting of flax, and is hence the most discussed and investigated enzyme concerning enzymatic retting [5, 20, 40–46, 21, 23–25, 31, 33, 34, 39]. Therefore, first combinations were designed with polygalacturonase (PAn). Polygalacturonase was combined with the five other enzymes, i.e. pectate lyase (Sc), pectin lyase (NS), pectin methylesterase (MPE), endoxylanase (Pz) and xylanase (XTI), as illustrated in Figure 2A. Next to polygalacturonase, xylanase can play an important role as well in the retting process [18, 24, 31–33, 41, 47–49]. Xylanase (XTI) was thus combined with the three important pectinase activities: pectate lyase (Sc), pectin lyase (NS) and polygalacturonase (PAn) (see Figure 2B). Pectin methylesterase was not combined with XTI because MPE will not be able to degrade the pectin backbone without the presence of another pectinase. Pz was not combined with XTI either, since these enzyme preparations exhibit a similar enzyme activity.

Next to the combinations with two enzymes, combinations with three enzymes were made and for this, PAn+MPE was taken as starting point due to the complementary activities. PAn+MPE was combined with the other pectinase activities, pectate lyase (Sc) and pectin lyase (NS), and with the xylanases, Pz and XTI (Figure 2C). Finally, two additional combinations were tested by combining the two main pectinase activities with a xylanase: PAn+Sc+XTI and PAn+NS+Pz (Figure 2D). Each enzyme was added to the enzyme formulation with 0.30 v/v % (in case of PAn 0.60 v/v %).

The abovementioned enzyme combinations were evaluated for their retting performance on flax by evaluating the resulting fiber fineness and mechanical properties of epoxy composites reinforced with the extracted fibers.

3.2 Fineness of fibers

Determination of the diameter of the extracted fiber provides further insights on the effect of the enzymatic treatment. When pectinases and hemicellulases are combined, the polymer network surrounding the elementary fibers within the technical fiber bundle should be degraded more thoroughly, resulting in a finer fiber. Results of analysis of fiber fineness with mean, median and maximum values are shown in Table 1, along with percentages of fiber widths higher than 300 μm and smaller than 100 μm . In addition, comparison is made with several reference materials and fibers from single enzymatic treatments. Distribution curves of selected fibers are illustrated in Figure 3.

Table 1. Results of analysis of fiber fineness with mean, median and maximum value as well as percentages of fiber widths higher than 300 μm and smaller than 100 μm

Treatment	Mean (μm)	Median (μm)	Maximum (μm)	% > 300 μm	% < 100 μm
References					
GR *	218	169	974	26.4	27.6
WATER *	114	106	614	2.2	49.7
EDTA *	113	85	614	1.9	50.2
DRm *	140	106	762	8.8	46.2
DRs *	116	106	508	1.8	47.3
FT *	83	64	551	0.2	68.9
Pectinases					
Sc *	167	148	953	9.7	26.2
Ns *	145	127	741	6.2	33.6
MPE *	152	148	635	6.5	30.1
PAn *	152	127	741	8.0	33.1
Hemicellulases					
Pz *	157	148	699	8.6	32.9
XtI *	173	148	805	11.5	27.6
Combinations with two enzymes					
PAn+Sc	130	106	974	5.7	44.7
PAn+NS	147	127	741	6.6	34.8
PAn+MPE	137	127	699	4.8	39.5
PAn+Pz	139	127	1038	6.5	39.4
PAn+XtI	163	148	1059	10.0	32.5
Sc+XtI	154	127	953	9.5	34.4
NS+XtI	142	127	889	9.3	40.9
Combinations with three enzymes					
PAn+MPE+Sc	124	106	635	3.1	44.0
PAn+MPE+NS	150	127	783	6.1	32.4
PAn+MPE+Pz	148	127	805	9.3	40.9
PAn+MPE+XtI	111	106	720	1.4	49.3
PAn+Sc+XtI	112	84	699	1.9	51.2
PAn+NS+XtI	158	127	783	10.7	35.9

* Data adapted from [25]

The starting material, i.e. green flax fibers denoted by GR in Table 1 possessed a mean fiber diameter of $218\ \mu\text{m}^1$ [25]. This fiber width is rather fine, since under-retted fibers, i.e., fibers that are not fully dew retted, have been reported with average fiber widths of 367 to 441 μm [50]. Manually extracted dew retted fibers (DRm) used as reference in this study showed a mean fiber diameter of 140 μm , while scutched dew retted fibers (DRs) possessed an average of 116 μm [25]. Water and EDTA treatment resulted in fibers with a mean fiber width on the same level as scutched dew retted fibers, 114 and 113 μm , respectively [25]. The distribution curve of manually extracted dew retted fibers is illustrated in Figure 3A.

¹ The mean fiber width of green fibers can be assigned to the manual extraction procedure. Mechanical scutching would probably break the green fibers, leading to almost no fiber yield. In order to increase this yield, a less extensive scutching step is necessary, leading to less fiber extraction. This implies very coarse fibers are obtained and lots of shives are left on the surface of the extracted fibers.

Figure 3. Distribution curves of fiber width (μm) of (A) manually extracted dew retted fibers (DRm) and fibers extracted after treatment with (B) PAN, (C) Sc, (D) NS, (E) XTI, (F) PAN+Sc, (G) NS+XTI and (H) PAN+Sc+XTI

Single enzyme treatments resulted in extraction of fibers with a fineness of 145 to 173 μm [25] and were hence finer than green fibers and similar to DRm fibers. The percentage of fibers smaller than 100 μm were however lower than for DRm fibers. Distribution curves of the single enzyme treatments PAn, Sc, NS, and XTI are illustrated in Figure 3, from B to E, respectively.

In comparison with the single enzyme treatments, treatments with combinations of two enzymes showed somewhat lower mean fiber diameters. Mean fiber widths were reduced after PAn+Sc, PAn+MPE, PAn+Pz, and NS+XTI treatment to 130, 137, 139 and 142 μm , respectively. These combinations clearly had more effect on the surrounding network of the fibers, which was also represented by the percentages of fibers smaller than 100 μm . The percentages were increased to 44.7, 39.5, 39.4, and 40.9 %, respectively, being closely in line with the percentage for DRm fibers (46.2 %) and being markedly higher compared to green flax fibers (27.6 %). Methyl groups will be cleaved by MPE while PAn can then degrade the non-methylated polygalacturonic acid. Sc acts on the same substrate as PAn, but these two enzymes were however also combined. NS can directly degrade methylesterified pectins. By combining PAn or NS with XTI or Pz, also hemicelluloses are degraded.

The combinations with three enzymes showed further improvements concerning fiber fineness. PAn+MPE+Sc treatment showed a reduced fiber width (124 μm), a similar percentage of fibers smaller than 100 μm in comparison with combinations with two enzymes but a lower percentage of fibers higher than 300 μm . Reduction of the percentage of fibers higher than 300 μm is very important since these are the fibers that will break during further processing. PAn+MPE+XTI and PAn+Sc+XTI treatments clearly had the most effect on fiber fineness with resulting mean fiber widths of 111 and 112 μm , increased percentages of fibers smaller than 100 μm of 49.3 and 51.2 % and very low percentages of fibers higher than 300 μm , i.e. 1.4 and 1.9 %, respectively. The latter results are hence better than green fibers and

manually extracted dew retted fibers. Comparable results were found for water and EDTA treatment when looking to the resulting fineness of the fibers and distribution curves.

Distribution curves of PAn+Sc (Figure 3F), NS+XTI (Figure 3G) and PAn+Sc+XTI (Figure 3H) showed a distribution similar to the manually extracted dew retted fibers and hence, a higher percentage of smaller fibers.

The lowest mean fiber width, along with the highest percentage of small fibers and lowest percentage of fibers bigger than 300 μm can be appointed to FlaxTape fibers. The high degree of fineness was reached as a result of the hackling step in the mechanical extraction process and cannot be accomplished by only manual extraction without any further refining steps.

Assessment of fiber fineness showed a clear improvement in average fiber width and percentage of small fibers when flax was treated with most mixtures of two enzymes. For the studied systems with three enzymes, an even higher enhancement was observed after PAn+MPE+XTI and PAn+Sc+XTI treatment, which showed competitive results compared with DRs fibers.

3.3 Tensile properties of composites

Good mechanical properties of flax fiber reinforced composites is of major importance to be able to implement these types of biocomposites in industrial applications and to encourage the use of eco-friendly materials. The assessment of tensile properties of the unidirectional composites is hence indispensable, along with the characterization of transverse properties. The Young's modulus of the composites was determined in different strain ranges: E1, the stiffness between 0.0 and 0.1 % strain and E2, the stiffness between 0.3 and 0.5 % strain, of which the results are illustrated in Figure 4A. Values were normalized for each composite specimen according to a 50 % fiber volume fraction for comparison

taking into account possible deviations in dimensions of the different composite samples. Back-calculated stiffness for fibers is shown in Figure 4B.

Figure 4. Normalized longitudinal E1- and E2-modulus of (A) composites impregnated with reference fiber materials and with fibers extracted from combined enzyme treated flax (GPa) and (B) back-calculated values for fibers (GPa)

* Data adapted from [25]

The E1-modulus of green fiber based composites was 39.1 ± 0.7 GPa [25]. Reference composites with water treated fibers, EDTA treated fibers, DRm fibers and DRs fibers showed E1-moduli that were not

significantly different from the E1-modulus from green fiber composites. The FlaxTape-epoxy composite did show a significantly lower E1-modulus of 31.1 ± 2.7 GPa ($P < 0.001$) [25]. Tensile properties of single enzymatic treatments, reported by De Prez et al. [25], showed E1-moduli between 41.4 and 43.9 GPa and were not significantly different from each other ($P > 0.05$). Compared to green fiber composites, significantly higher E1-moduli were observed for composites reinforced with fibers extracted after Sc and XTI treatment ($P \leq 0.002$) [25].

Fibers extracted after flax treatment with combinations of two enzymes resulted in fiber-epoxy composites with E1-moduli from 42.6 to 44.2 GPa. Compared with green fibers, fibers after each combination with two enzymes resulted in composites that showed significantly higher E1-moduli ($P < 0.05$), with exception of fibers after NS+XTI treatment. The two enzyme combinations were not significantly different from each other concerning E1-moduli, nor were they significantly different from the single enzyme treatments and water and EDTA treatments. Compared to the scutched dew retted fibers, enzymatically treated fibers resulted in composites with significant higher stiffness results.

Flax treatment with the defined combinations of three enzymes produced fiber composites with an E1-modulus ranging from 41.7 to 43.9 GPa. Good tensile properties were noticed for the different enzymatically treated fibers being thereby very competitive with the classical dew retted fibers. Again no significant differences were found between enzymatic treatments. The modulus is however a bulk property, representing an average of all included fibers in the composite material. Among the combinations with three enzymes, only PAn+MPE+Pz treatment provided fiber composites with a significantly higher E1 modulus compared to green fiber composites. The similarity in results between single and combined enzymatic treatments could imply that environmental factors like temperature, fiber to liquid ratio and presence of EDTA could play a more important role than previously anticipated, since

water and EDTA treatment of flax also led already to high-quality composites. By adding EDTA, calcium is removed from the epidermis, which enhances the retting efficiency [5].

As observed for the single enzymatic treatments [25], also preservation of the stiffness was successful for composites reinforced with fibers after combined enzyme treatments. An E2/E1 ratio could be found between 92 and 95 %, while in literature, drops of 31 % have been observed for unidirectional flax-epoxy composites [25, 37, 50, 51].

In relation to fineness, it is expected that in case of similar fiber properties, finer fibers would result in a composite with higher stiffness. However, the lowest Young's modulus is observed for the composites impregnated with FlaxTape, which strangely possesses the lowest mean fiber width as well. In research it was observed that coarser fibers were linked with higher stiffness results [52]. Other research showed that longitudinal properties of composites are not very sensitive to a change in fiber stiffness [50], which can be confirmed by the results in this study.

Back-calculated fiber moduli were determined by applying the rule of mixtures and are shown in Figure 4B. Mechanical properties of fibers were not characterized by mechanically testing the technical or elementary fibers as such, since research has shown that no direct correlation exists between (technical or elementary) fiber and composite properties [5, 35, 53, 54]. For flax treated with combinations with two enzymes, back-calculated E1-moduli for fibers were obtained from 85.2 ± 3.0 GPa (NS+XTI) to 88.4 ± 4.3 GPa (PAn+Pz). Combinations with three enzymes resulted in fibers with an E1-modulus of 82.5 ± 3.1 GPa (PAn+Sc+XTI) to 87.7 ± 4.4 GPa (PAn+MPE+Pz), while dew retted fibers showed an E1-modulus around 70 to 76 GPa. Bourmaud et al. [4] reported a Young's modulus for flax fibers within a range of 37.2 to 75.1 GPa [12, 27–30], while Pickering et al. [10] described a range from 27 to 80 GPa. Joffe et al. [55] reported flax fibers with an average Young's modulus of 89 ± 35 GPa. Hence, in this PhD-study very high Young's

moduli were observed for technical fibers. Compared to glass fibers, which have a Young's modulus of approximately 70 GPa [10, 56], a definite improvement has been found which is an important contribution towards a future biocomposite industry.

Tensile testing of composites also delivers the tensile strength, as illustrated in Figure 5A. Back-calculated fiber tensile strengths are included as well (Figure 5B). Composites based on the starting material, i.e. green fibers, showed a longitudinal tensile strength of 404 ± 26 MPa [25]. DRs and FT fibers resulted in significantly lower tensile strengths of 315 ± 15 MPa and 324 ± 32 MPa ($P \leq 0.01$), respectively [25]. The low tensile properties observed for scutched dew retted fibers and FlaxTape fibers may be attributed to the extensive mechanical treatment, which caused fiber damage. The manual extraction method on the other hand seemed to preserve fiber properties without inducing damage. Nevertheless, despite the induced damage due to mechanical extraction, it has been observed in other research that the further refining of the hackling step can compensate for the induced damage, leading to high tensile strengths nonetheless [50, 53, 57]. This is clearly not the case in this study. The induction of damage by mechanical extraction however results in the origin of more kink bands and can have its effect on the compressive properties of the composite, but these properties are not determined in this study. Concerning the single enzyme treatments, tensile strengths of 396 to 421 MPa have been measured for composites but were not significantly different amongst each other or in comparison with GR, water, EDTA and DRm fiber composites [25].

Treatment with combinations of two enzymes resulted in fiber composites with tensile strengths varying between 424 and 449 MPa. Again, no significant differences could be found. For three enzyme combinations, composites were reported with tensile strength ranging from 408 to 449 MPa. Between all

enzymatic treatments, i.e. single treatments, combinations with two and three enzymes, no significant differences could be found concerning the tensile strength of composites.

Figure 5. Normalized longitudinal tensile strength of (A) composites (MPa) to a fiber volume fraction of 50 % and (B) back-calculated fiber tensile strength (MPa) * Data adapted from [25]

Back-calculated fiber tensile strength amounted to 776 ± 50 MPa in case of green fibers and increased to 891 ± 59 MPa for fibers after water treatment ($P > 0.05$) [25] (Figure 5B). A significant difference was found for DRs and FT fibers ($P < 0.01$), with tensile strengths of 602 ± 30 and 611 ± 60 MPa, respectively

[25]. Single enzymatic treatments showed no significant differences in back-calculated fiber strengths [25]. As concluded for the composite tensile strengths, no significant differences could be found in back-calculated fiber tensile strength for all enzymatic treatments. Fiber strengths of 788 to 865 MPa were reported after enzymatic treatment with two or three enzymes. In contrast, in literature fiber tensile strengths have been observed from 595 to 1510 MPa [4, 12, 28, 30]. These high tensile strengths were obtained for elementary fibers and shorter gauge lengths and can therefore not be compared with the results of this study. In research of Bos et al. [58], elementary fibers with a tensile strength of 1500 to 1800 MPa were found, but only a span length of 3 mm was used.

When different enzymes or enzyme combinations are applied on flax, again similar but high tensile strengths are observed for the reinforced composites. It could be possible that pectinase and/or hemicellulase activity rearranges or degrades the surrounding network of the fiber in such a way that a strong bond can exist between fiber and matrix in the interface. Removal of pectin loosens the fibers from the woody core, hereby facilitating fiber extraction. However, the presence of pectin around the fiber is also shown to be beneficial when impregnated in composite materials for the quality of the interface and the transfer of applied loads between fiber and matrix and between fibers within the fiber bundle [5, 53]. Question rises if water as such is sufficient to loosen the pectin network around the fiber, since it is already known that water retting delivered high-quality fibers, which is also confirmed by the results in this study. The addition of EDTA can help in the degradation of pectin, which can be seen by the promising results of fibers extracted after EDTA treatment. The lack of distinction between different enzymatic treatments may imply that water and EDTA are more important than previously anticipated for.

3.4 Transverse properties of composites

For full characterization of mechanical properties, also the transverse bending strength of composites reinforced with fibers extracted after enzymatic treatments were determined (Figure 6).

Figure 6. Transverse bending strength [MPa] of composites reinforced with reference fiber materials and fibers extracted after combined enzyme treatments * Data adapted from [25]

Green fiber-epoxy composites were characterized with a transverse bending strength of 17.2 ± 2.3 MPa. Compared with the green fiber composites, composites reinforced with fibers after water treatment, fibers after EDTA treatment, DRm fibers and fibers extracted after Sc treatment showed no significant difference in transverse bending strength ($P > 0.05$) [25]. Even though tensile properties were less promising, FlaxTape shows most promising composite transverse bending strengths (40.9 ± 1.6 MPa) compared to the other references and enzymatically treated fibers. The hackling step further refines and aligns the fibers. FlaxTape shows indeed the lowest average fiber width of $83 \mu\text{m}$. The high transverse flexural strength of the FlaxTape composite can thus probably be correlated with the high fineness or by extension the high purity. More fiber surface can be exposed to the matrix material implying less inter-

elementary fiber interphases are present within the technical fibers. If fibers are subjected to an additional hackling step, the weakest of these interphases are eliminated, leading to fibers with better transverse properties. Furthermore, the high degree of alignment of FT fibers may have had a positive impact on the transverse bending properties as well. Fineness accompanies purity, and fewer impurities on the fiber surface will imply a better fiber-matrix interface [50], even though better fineness can mean increased fiber damage as well. In research of Hendrickx et al. [35], unidirectional FlaxTape-epoxy composites have been reported with a transverse bending strength of 37 MPa, and this is in line with our result.

Single enzyme treatments were reported to result in composites with a transverse bending strength ranging from 21.3 to 25.4 MPa and were not significantly different amongst each other [25]. Combinational treatment with two enzymes resulted in fiber composites with bending strengths from 22.0 to 24.5 MPa, while combinations with three enzymes produced composites with transverse strengths between 22.1 and 23.6 MPa. Hence, no significant differences could be found between all single or combined enzymatic treatments, but a comparable quality is obtained in comparison with scutched dew retted fibers. Differences between fiber fineness of enzymatically treated fibers were probably not large enough to observe significant differences in transverse bending strength of the composites or the similarity in results could also imply that environmental factors like temperature, fiber to liquid ratio and presence of EDTA could play a more important role than previously anticipated, since water and EDTA treatment of flax also led to high-quality composites.

3.5 Fracture surface analysis of composites after bending tests

Composite specimens subjected to three point bending tests were analyzed using SEM. A schematic representation of the composite specimen subjected to the three-point bending test is shown in Figure 7.

Figure 7. Schematic representation of composite specimen subjected to three-point bending test

The arrow shows the location where the fracture surface was analyzed using SEM. The fracture surface was visualized for composites reinforced with fibers extracted after PAn+Pz (Figure 8A and B) and PAn+Sc treatment (Figure 8C and D) as illustration.

The SEM images indicate that the fracture surfaces of composites with fibers after PAn+Pz treatment show a higher degree of fibrillization or more single technical fibers compared to the fracture surfaces of composites with fibers after PAn+Sc treatment. This however cannot be confirmed by the fineness results. Further fibrillization however can occur due to failure, definitely at the fracture surface. In Figure 8A and B, failure of a single technical fiber can be observed in the fracture surface of the PAn+Pz fiber composite. For PAn+Sc treatment however, in Figure 8C and D, fiber bundles are less loosened and more coherent. Nevertheless, no differences could be found in tensile properties or transverse properties for both treatments. The same observation was made for the other treatments. This indicates that the fiber-matrix tensile adhesion strength is in the same order of magnitude as the relatively low transverse strength of

the technical fibers, meaning that small differences in either of the strength values lead to a clear difference in failure mode.

Figure 8. SEM analysis of fracture surfaces of transverse three-point bending test composite samples impregnated with fibers extracted after treatment with (A) PAn+Pz (100x) and (B) 80x, (C) PAn+Sc (103x) and (D) 99x. The scale bar represents 200 μm

4. Conclusions

To investigate the effect of strategic enzyme combinations on flax, fiber fineness and mechanical properties of fiber-epoxy composites was studied. Pectinases and hemicellulases play an important role to degrade pectin and hemicellulose in the surrounding network of the fiber. Strategic enzyme combinations were based on the key activities polygalacturonase, xylanase and the combination of polygalacturonase + pectin methylesterase.

PAn+MPE+XTI and PAn+Sc+XTI treatments exhibited the highest effect on fiber fineness with average fiber widths of 111 and 112 μm , increased percentages of fibers smaller than 100 μm of 49.3 and 51.2 % and very low percentages of fibers higher than 300 μm , i.e., 1.4 and 1.9 %, respectively. These results are competitive with scutched dew retted fibers, although water and EDTA treatment also resulted in fine fibers. FlaxTape fibers showed the highest percentage of fibers smaller than 100 μm of 68.9 % and 0.2 % of the fibers had a fiber width bigger than 300 μm , which can be ascribed to the additional hackling of the flax fibers.

Mechanical properties showed no significant differences in tensile or transverse properties between the different combined enzymatic treatments. This similarity in results could imply that environmental factors like temperature, fiber to liquid ratio and presence of EDTA could play a more important role than previously anticipated, since water and EDTA treatment of flax also led to high-quality composites. FlaxTape showed most promising composite transverse bending strength ($40.9 \pm 1.6 \text{ MPa}$) compared to the other references and enzymatically treated fibers. It is important however to address that enzymatically treated fibers were manually extracted in contrast to FlaxTape which was scutched and hackled. Therefore, future research should be focused on the investigation of the mechanical processing necessary after the enzymatic treatment and to evaluate the effect on the composite properties. Hereby, more damage will be induced but fibers will also be further refined which will affect the mechanical properties as has been demonstrated for FlaxTape. To gain these further insights, the enzymatic treatments must be first carried out on bigger scale.

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Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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