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Treating bast fibres with pectinase improves mechanical characteristics of reinforced thermoplastic composites

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Abstract

The effect of pectinase treatment of hemp (*Cannabis sativa* L.) fibres on mechanical properties of fibre reinforced polypropylene was investigated. Incubating technical fibres with pectinase (SIHA-Panzym[®] DF) reduced their cross-sectional area ($8.72 \cdot 10^{-3}$ vs. $3.67 \cdot 10^{-3}$ mm², control vs. pectinase incubation) and decreased tensile strength (455.4 vs. 171.2 MPa). Polypropylene (PP) was reinforced with these fibres using conventional extrusion and injection moulding techniques. Maleic anhydride grafted PP was applied as compatibilizer. The cross-sectional area of fibres in the composite decreased from $2.58 \cdot 10^{-4}$ to $1.21 \cdot 10^{-4}$ mm² for control vs. pectinase. Tensile strength (43.7 vs. 47.6 MPa for control vs. pectinase incubation), flexural strength (49.9 vs. 56.6

MPa) and moduli of elasticity (tensile, 2.62 vs. 3.25 GPa and flexural, 2.28 GPa vs. 2.87 GPa, respectively) all increased when using fibres incubated in pectinase. Qualitatively and quantitatively similar effects, albeit on a decreased level, were obtained when reinforcing PP in absence of the compatibilizer. The data indicate the refining of technical fibres in response to pectinase treatment. This effect was accomplished by (1) enzymatic decomposition of the middle lamellae, (2) mechanical stress exerted by compounding and (3) compression of cell lumina. The separation of technical fibres into smaller bundles and single fibre cells, in turn, resulted in improved tensile and flexural characteristics of thermoplastic composites.

Keywords: A. Short-fibre composites; B. Mechanical properties; D. Environmental scanning electron microscopy (ESEM); E. Extrusion; Injection moulding

1. Introduction

In the past decade mechanical properties, processing technologies and the interfacial compatibility of cellulose fibre reinforced composites were investigated extensively [1,2,3,4]. Using vegetable fibres in reinforced thermoplastics and thermosets has a number of technological and environmental benefits [5]. Vegetable fibres are obtained from a range of sources, including stem (bast) and leaf fibres from flax, hemp, jute, ramie, sisal and other fibre crops. In the plant body fibres provide structural support by absorbing mechanical stress. By using these fibres in reinforced composites key properties of plant fibres are transferred to the composite: tensile strength and stiffness similar to those of E type glass fibres [1], low density (range 1.15 to 1.6 g·cm⁻³) similar to aramide fibres (1.4 g·cm⁻³ [2]). Additional benefits result from low energy

consumption and CO₂ pollution in processing as well as from recyclability. However, for advanced technical applications vegetable fibres have to be prepared regarding (i) the chemical and physical homogeneity of fibre batches, (ii) the degree of degumming and elementarization of fibre bundles, (iii) the degree of polymerization and crystallization, (iv) the adhesion between fibres and matrices, (v) the moisture repellence, and (vi) the flame retardant properties [1].

Technical fibres are coarse and inhomogeneous when extracted mechanically from hemp straw using industrial scale decortication equipment. The quality of these fibres may be considerably improved when decortication is followed by further refining treatments. Here we focused on incubation with pectinase that is expected to decompose the middle lamellae selectively and to separate technical fibres into smaller bundles and single fibre cells. The objective of the present study, therefore, was to establish the effect of pectinase treatment on the physical properties of hemp fibres and on the mechanical characteristics of a model composite consisting of these fibres and polypropylene.

2. Experimental

2.1. Plant material

Hemp (*Cannabis sativa* L. cv. Kompolti) for fibre production was grown according to the current agricultural practice (seeding date: 25 April; seeding density: 450 m⁻²; N supply: 120 kg·ha⁻¹; no measures for growth regulation and crop protection; harvest date: 8 September) at the Experimental Station of the Martin-Luther-Universität Halle-Wittenberg in Leipzig-Seehausen (51°21'N, 12°25'E; Germany). Stands were harvested with a combine at 10 to 20 cm above the ground surface and cut to 60 to 80 cm length.

Stalks remained in a swath for dew retting until a medium degree of retting was attained. The stalks were baled (19 October). Fibres were extracted mechanically using a custom build roller device equipped with rollers for breaking stalks, rotating sieves for separating shives and further refining units (LaRoche, Vernaro GmbH, Gardelegen, Germany). The product obtained by this process is referred to as technical fibres. In botanical terms technical fibres represent fibre bundles consisting of numerous fibre cells. Unless stated otherwise we use the term fibres for fibre bundles and for those filamentous products arising from pectinase treatment (see also [6]).

2.2. Enzyme treatment, composite manufacture

Hemp fibres were cut into shorter segments of 8 mm length, and incubated in 8% (v/v) SIHA-Panzym[®] DF (EC 3.2.1.15, Novozyme A/S, Bagsvaerd, Denmark) in 1 M sodium hydrogen carbonate, 1 M citric acid, 10 mM ethylenediaminetetraacetic acid (EDTA) at 35°C and pH 4.5. EDTA was added to the incubation medium to destabilize pectins by complexing cell wall bound calcium [7,8,9]. After 4 h fibres were removed from solution, rinsed twice using tap water, centrifuged, and air-dried for 2 to 3 d. There were two independent preparations (prep1, prep2). Non-treated fibres served as controls. For physical characterization of fibres a subsample was taken before milling (SM 2000, Retsch GmbH, Haan, Germany) and sieving using a sieve of 2 mm pore size.

For compounding a co-rotating twin-screw extruder (ZSE 27 GL-44D, Leistritz Extrusionstechnik GmbH, Nürnberg, Germany) was used. The temperature gradient was set at 190°C and 160°C between heating zones 1 and 10. Polypropylene (Atofina PPH 7060, Total Petrochemicals, Felny, Belgium) and hemp fibres were fed in heating zones 1 and 6 of the extruder, respectively. For fibres, a FlexWall[®]Plus feeder (DDW-MD-

FW40/4plus-ISC, Brabender Technologie KG, Duisburg, Germany) and a twin-screw volumetric feeding system (T-35, K-Tron AG, Niederlenz, Switzerland) was used. In some batches 2% (w/w) maleic anhydride modified polypropylene (SCONA TPPP 7112 FA, Kometra GmbH, Schkopau, Germany) was added as a compatibilizer. Following cooling strands were pelletized to 3 mm length (SP 30, Pell-Tec Pelletizing Technology GmbH, Niedernberg, Germany).

2.3. Analyses

Physical characteristics of fibres were determined on subsamples obtained prior to milling. Fibres free of splices and splinters were used as test specimen. Thickness was recorded along a 45-mm part of the specimen at a 90° angle using a two-axial laser micrometer (ODAC 15Y-JM, Zumbach, Orpund, Switzerland). As a first approximation cross-sectional area of fibres was estimated assuming a cylindrical shape of the specimen and using mean thickness as mean diameter of a circle. Tensile tests were performed using a universal instrument for material testing (model 1446, Zwick AG, Ulm, Germany). Gauge length, initial load and test velocity were set at 2 mm, 0.05 N and 3 mm·min⁻¹, respectively. Test parameters were calculated using the software TestXpert 6.0 (Zwick AG, Ulm, Germany).

Mechanical characteristics of the composite were established according to the standards DIN EN ISO 527-1 and 2 (tensile test) and DIN EN ISO 178 (flexural test) on a universal test instrument (Z010/TH2S, Zwick AG, Ulm, Germany). Specimens were prepared on an injection moulding machine (Allrounder 320 C 500-250, Arburg GmbH & Co KG; Lossburg, Germany) at a maximum temperature of 180°C and an injection

pressure of 160 MPa. Impact testing was carried out with the Pendelschlagwerk 5.102.102 (Zwick AG, Ulm, Germany) according to the standard DIN EN ISO 179.

For microscopy compound pellets (prep1) were cut with the help of a diamond saw. Surface was ground and polished using 30, 9, 6, and 1 μm -diamond polishes. Surface images were obtained from environmental scanning electron microscopy (XL30, ESEM-FEG, Philips Eindhoven, Netherlands). Cross-sectional areas of fibres on the microscopic images were measured using the imaging software Analysis 3.0 (Soft Imaging Systems GmbH, Münster, Germany)

2.4. Statistics

Analysis of Variance (ANOVA) and bootstrap analysis were performed using SAS Systems for Windows 8.02 (SAS Institute Inc., Cary, USA) and 2SAMPLE [10] (software free available from Mudelsee, University of Leipzig, Germany; <http://www.uni-leipzig.de/~meteo/MUDELSEE/>), respectively. All statistical tests were carried out at $P = 0.05$. For ANOVA, data on fibre characteristics (cross-sectional area, tensile strength) were transformed using the cubic root to obtain normal distributions. Data are displayed as box-whisker plots with box, whiskers and crosses giving the 25th to 75th, 10th to 90th and 5th to 95th percentiles, respectively. The horizontal line in the box shows the median. The numerical data given above the boxes represent the back-transformed arithmetic mean. Bootstrap comprised 1999 simulations. Significant median differences were stated when the 95%-confidence interval did not include zero.

3. Results

Incubating technical hemp fibres in pectinase significantly decreased cross-sectional area by 42 and 46% in two independent preparations, respectively (Fig. 1A; prep1 and prep2). Furthermore, tensile strength of fibres significantly decreased by 37 and 52% (Fig. 1B). Both statistical procedures, i.e., ANOVA (on transformed data) and bootstrap (on non-transformed data; analysis not shown), yielded the same conclusion.

In the hemp fibre polypropylene composite (HFPP) fibres incubated in pectinase significantly increased tensile strength and tensile modulus of elasticity regardless of the absence and presence of the compatibilizer maleic anhydride (\pm MA) (Table 1). Ultimate elongation either decreased or was not affected by pectinase treatments. There was no consistent effect of pectinase on elongation at break. Flexural strength and flexural modulus of elasticity increased markedly in composites with fibres from pectinase treatments. Further, there was no consistent effect of pectinase treatment on Charpy impact strength of the composite (Table 1). In prep1, but not in prep2 Charpy impact strength was increased (control: HFPP-E \pm MA), when fibres were incubated in pectinase (HFPP+E \pm MA).

Comparison of all pectinase and maleic anhydride effects revealed that the highest tensile and flexural strengths and the corresponding moduli of elasticity were generally obtained in composites containing pectinase treated fibres and maleic anhydride (HFPP+E+MA). Furthermore, effects of pectinase on tensile strength were smaller than those of MA (2.1 to 8.9% vs. 29.7 to 34.8% for pectinase vs. MA). However, effects of pectinase on flexural strength were larger than those of MA (13.4 to 34.4% vs. 4.8 to 24.1% for pectinase vs. MA). Thus, flexural properties were more affected by pectinase than by MA, but the reverse was true for tensile properties.

Micrographs obtained by ESEM of HFPP pellets showed a deformed structure of embedded fibres (Fig. 2AB), that did not allow identification of individual fibre cells. Cell lumina were mostly collapsed and cell walls appeared condensed with numerous cracks along middle lamellae of fibre bundles or across cell wall layers of fibre cells resulting in numerous fragments. The orientation of the fibres in the PP matrix appeared randomized. Cross-sectional areas of fibres differed significantly between non-treated control ($2.58 \cdot 10^{-4} \text{ mm}^2$) and pectinase treatments ($1.21 \cdot 10^{-4} \text{ mm}^2$; Fig. 2C). It is interesting to note that cross-sectional area of fibre cells in the composite were about ten-fold smaller than those determined prior to processing by milling and compounding (see Fig. 1A). In absence of MA (HFPP-MA) fibres were often surrounded by wide gaps that prevented contact between fibre and PP matrix (Fig. 2A). These gaps were not apparent in samples with MA grafted PP (HFPP+MA; Fig. 2B).

4. Discussion

4.1. Fibre refining

Bast and leaf fibres represent elongated sclerenchyma cells that are assembled in bundles forming a part of the phloem. In the plant fibre cells are surrounded by thin walled tissues, e.g., sieve elements, companion cells, parenchyma of rays, bark and mesophyll. Mechanical decortication equipment breaks the thin cell walls of the latter structures thereby releasing fibre bundles of varying dimensions (technical fibres).

Residual cell wall fragments remain on the surface of the fibre bundles leaving behind a pattern of imprints of the neighbouring cells. To improve hemp fibre quality additional refining procedures are required, i.e., cleaning the surface and splitting fibre bundles into finer units, ideally into individual fibre cells. For refining various methods may be

adapted including steam explosion [11], ball milling of thermally treated fibres [12,13,14], chemical degumming in alkaline media [15,16], enzymatic separation of technical fibres, and scouring [17,18].

Alternatively, the stalks of fibre plants may be subjected to complex microbial and enzymatic decomposition processes referred to as retting. Effective retting allows the isolation of fibre bundles of reduced thickness [19]. Retting organisms release cell wall degrading enzymes including pectinases. This group of enzymes comprises polygalacturonases, pectin esterases, pectin lyases and pectate lyases. Pectinases are also used in biotechnological processes [20]. In particular the alkaline forms are often applied for degumming of vegetable fibres [21]. Chelators support fibre refining by retting [22,23,24]. However, cellulase activity may be associated with retting and degumming agents. Cellulases corrode the cellulose of the primary and secondary cell wall layers particularly, at the nodes and dislocations of the fibres [24,25].

We focussed on pectinase treatment of mechanical decorticated hemp fibres.

Preliminary data established that SIHA-Panzym[®] DF was the most effective for separating technical hemp fibres into smaller bundles and single fibre cells among thirty preparations investigated (data not shown). It represents a blend of pectinases developed for must processing in the wine industry. As expected, SIHA-Panzym[®] DF also degraded the middle lamellae of hemp fibre as indexed by a markedly decreased cross-sectional area of fibres (Fig. 1A). Since cross-sectional area of fibre is closely related to fineness [26], pectinase treated fibres should have a low fineness. Microscopy of fibres revealed that imprints of neighbouring cells disappeared upon incubation in pectinase (data not shown) analogous to scouring. [18].

The structural changes induced by the pectinase treatment altered the mechanical properties of the fibres as indicated by decreased tensile strength (Fig. 1). This effect is primarily accounted for by loosening of the pectin lamellae. Clearly, side effects of residual cellulytic activity in the SIHA-Panzym[®] DF preparation cannot be excluded. However, there was no evidence from microscopy in support of the latter activity. Similar findings are reported on bioscouring of hemp fibres [18]: Pectate lyase (Scourzyme L from Novozyme, Australia) smoothed the fibre surface, separated fibre cells, removed pectin and increased the surface area as well as the pore size. However it also decreased tensile strength and E-modulus when incubation was extended to more than five hours.

4.2. Composite characteristics

HFPP prepared using pectinase treated fibres exhibited improved tensile and flexural characteristics (Table 1). These effects may be attributed to the refinement resulting in separation of technical fibres into smaller bundles and single fibre cells. The decrease in tensile strength of the fibres did not translate into decreased tensile strength of the composite (Fig. 1 vs. Table 1). This effect may be surprising at first sight. However, refining fibres increased the surface to mass ratio of the fibres leading to a markedly enlarged interfacial area between fibre and matrix. Support for this hypothesis comes from literature data: Flax fibre reinforced PP (\pm MA) exhibited a higher tensile and flexural strength when hackled (fine) instead of scutched (coarse) fibres were compounded [27]. Similarly, studies on wood fibre PP composites reported increased tensile and flexural properties as a result of an increased aspect ratio (length per cross-

sectional diameter) [28]. In addition the removal of pectins may provide a sound base for interfacial binding between cellulose and PP matrix [29].

It is interesting to note that despite the order of magnitude decrease in cross-sectional area of fibres during compounding the relative difference in fibre cross-sectional area between composites containing pectinase treated and non-treated fibres remained about the same (Fig. 1A vs. Fig. 2C). Clearly, this comparison is somewhat skewed, since orientation of fibres in the pellet was random and hence, apparent cross-sectional areas of fibres in the pellet are not representative for the true cross-sectional area of the fibre. Thus our estimates of fibre areas from the pellet are conservative underestimating the effect of compounding on area. This, however, applies equally to both non-treated and pectinase treated fibres. The absence of a consistent effect of pectinase treatment on impact strength may be attributed to the complex mechanistic basis comprising matrix fracture, fibre-matrix debonding, pull-out of fibres, and fibre fracture. The relative importance of these processes and the effect of pectinase treatment thereon are currently unknown.

The effect of pectinase treatment of fibres on mechanical characteristics of HFPP was independent of the compatibilizer MA. That MA was effective in our experiments is demonstrated by data in Table 1. Graft co-polymerisation of hot PP and MA results in a covalent and cohesive coupling of cellulose hydroxyl groups to the PP matrix (for summary see [1]). This effect is mechanistically different from those of the pectinase treatment discussed above.

5. Conclusion

In summary our data provide evidence for an effective refinement of hemp fibres when incubated with a commercially available pectinase. The use of pectinase treated fibres in reinforced PP significantly improved tensile and flexural characteristics of the composite.

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Table 1: Mechanical characteristics of hemp fibre reinforced polypropylene (HFPP).

Fibres were treated without (-E) and with pectinase (+E) before compounding in polypropylene without (-MA) and with 2% (w/w) maleic anhydride (+MA) grafted polypropylene. Data were obtained from two independent preparations (prep1, prep2). Different letters within preparations indicate significant difference of arithmetic means by ANOVA at $P=0.05$ ($n = 10$; * $n = 5$).

Fig. 1: Cross-sectional area (A) and tensile strength (B) of hemp fibres (HF) without (-E, contr) and with pectinase incubation (+E). Data for pectinase treated fibres were obtained from two independent preparations (prep1, prep2). Numerical data above boxes represent back-transformed arithmetic means. Different letters indicate significant difference of arithmetic means by ANOVA at $P=0.05$ (contr: $n = 104$; prep1, prep2: $n = 100$).

Fig. 2: Micrographs obtained from environmental electron microscopy (ESEM) of cross-sections of hemp fibre reinforced polypropylene (HFPP) pellets (A,B) and measurement of cross-sectional areas of hemp fibres in the composite (C). A: fibres without pectinase incubation (-E) compounded in polypropylene (PP) without maleic anhydride (-MA); B: fibres incubated in pectinase (+E) compounded in PP plus 2% (w/w) maleic anhydride grafted PP (+MA). Arrows indicate gaps between fibre and PP. C: fibres without (-E) and with pectinase incubation (+E) before compounding.

Numerical data above boxes represent back-transformed arithmetic means. Different letters indicate significant difference of arithmetic means by ANOVA at $P=0.05$ (-E: $n = 483$; +E: $n = 255$).

Table 1

Preparation	Sample	Tensile test				Flexural test		Impact test
		Tensile strength (MPa)	Tensile modulus of elasticity (MPa)	Ultimate elongation (%)	Elongation at break (%)	Flexural strength (MPa)	Flexural modulus of elasticity (MPa)	Charpy impact strength (kJ m ⁻²)
prep1	HFPP-E-MA	33.6 d	2215 d	4.08 a	5.46 a	40.2 d	2170 d	3.38 c *
	HFPP-E+MA	43.7 b	2615 c	3.87 b	4.40 b	49.9 b	2283 c	3.58 bc *
	HFPP+E-MA	35.7 c	3008 b	2.94 d	3.56 c	47.6 c	2983 a	3.73 b
	HFPP+E+MA	47.6 a	3246 a	3.78 c	4.29 b	56.6 a	2870 b	4.06 a
prep2	HFPP-E-MA	34.0 d	2728 d	3.74 a	4.58 a	41.5 d	2298 d	3.80 a
	HFPP-E+MA	44.1 b	3073 b	3.51 b	3.86 b	43.5 c	2657 b	3.81 a
	HFPP+E-MA	34.7 c	2981 c	3.70 a	4.75 a	53.2 b	2498 c	3.99 a
	HFPP+E+MA	46.8 a	3291 a	3.11 c	3.54 b	58.5 a	2971 a	3.25 b

Fig. 1

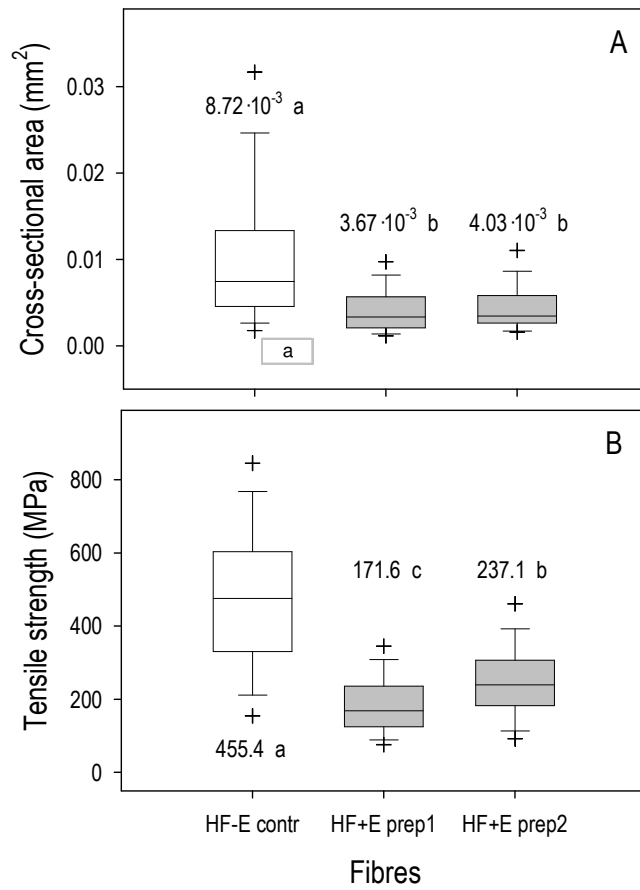


Fig. 2

