Preparation, Properties, Protein Cross-Linking and Biodegradability of Plasticizer-Solvent Free Hemp Fibre Reinforced Wheat Gluten, Glutenin, and Gliadin Composites

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The present study is aimed at evaluating the use of plant-based polymers and fibres for the production of sustainable biocomposites. For the first time, plasticiser/solvent-free hemp fibre-reinforced wheat gluten and hemp-gliadin and glutenin composites were obtained by compression moulding at different temperatures. The plasticiser/solvent-free sample preparation method developed in this study facilitated the use of a powdered protein matrix with a mat of randomly oriented hemp fibres. The tensile and protein cross-linking properties, as well as the biodegradability, were investigated. The addition of hemp fibre to the protein matrix increased the E-modulus by 20 to 60% at 130 °C. An increase in moulding temperature from 110 to 130 °C resulted in an increase in maximum stress due to the formation of intermolecular bonds between protein chains. The gliadin composites had higher E-modulus and maximum stress and showed a larger increase in protein polymerisation with increased temperature compared to the glutenin composites. A comparison of tensile properties revealed that the composites were stiffer and stronger compared to several similarly produced biobased composites. The composites were found to be fully biodegradable under a simulated soil environment after 180 days. Biocomposites produced in the present study were found to be environmentally friendly with fairly good mechanical properties.

Keywords: Wheat gluten; Hemp fibre; Biocomposites; Compression moulding; Tensile properties; Protein cross-linking; Biodegradability

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INTRODUCTION

The depletion of petroleum resources and the increasing demand for environmentally friendly plastic materials has resulted in a focus on research related to bio-based polymers and composites specifically from renewable resources (Kunanopparat *et al.* 2008a; Huang and Netravali 2009; Wretfors *et al.* 2009). Plant proteins, *e.g.*, wheat gluten (WG), are such a resource, being both inexpensive and widely available and thereby interesting as a biodegradable matrix for the production of biobased composites (Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; Reddy and Yang 2011a; Blomfeldt *et al.* 2012).

Wheat gluten has interesting properties, *e.g.*, good ability to form films, good mechanical performance, and high oxygen barrier properties (Olabarrieta *et al.* 2006). The major issues regarding the use of plant proteins are degree of cross-linking, water resistance, and ductility (Lagrain *et al.* 2010). Wheat gluten consists of high-molecular weight (HMW) and low-molecular weight (LMW) polymeric glutenins and monomeric gliadin, together with small amounts of starch, bran, and fibre. In the wheat grain or flour, the polymeric glutenin is responsible for intermolecular disulphide linkages, while monomeric gliadin contains only intra-molecular linkages (Wrigley *et al.* 1988; Wieser 2007). Under certain conditions, *e.g.*, during mixing, heating, and pressing, disulphide linkages are rearranged so that gliadin is incorporated in the polymeric proteins (Johansson *et al.* 2013; Kuktaite *et al.* 2004). Glutenin and gliadin proteins have been used separately for making thermoplastic films, and glutenin films demonstrated higher strength and modulus, but lower extensibility, compared to gliadin films (Chen *et al.* 2012; Rasheed *et al.* 2014).

Natural fibres from plants can be used as reinforcements for making bio-based composites. Examples of plant fibres which have been used to produce plastic composites are bamboo, flax, hemp, and jute (Bismarck *et al.* 2002). The addition of hemp, bamboo, or jute fibre in WG and soy protein-based composites yielded increased tensile strength and stiffness (Kunanopparat *et al.* 2008a; Huang and Netravali 2009; Wretfors *et al.* 2009; Reddy and Yang 2011a,b).

Efforts have been made to produce composites from WG and hemp fibres through compression moulding, using glycerol as a plasticiser (Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; 2010). Poor bonding between the WG matrix and the hemp fibres, observed as the phenomenon of "fibre pull-out" in the fracture surface, has been reported in previous studies (Wretfors *et al.* 2009; 2010). During processing, proteins in the matrix are polymerised, a process which depends on many factors, including temperature, pressure, pH, plasticiser content, type of matrix, reinforcement type, and processing method used to produce the composite material (Redl *et al.* 1999; Johansson *et al.* 2013). Hence, the mechanical properties of the composite depend not only on the presence of fibres but also on aggregation/cross-linking of the protein (Bismarck 2002; Kunanopparat *et al.* 2008a). Biodegradation analyses have shown that WG-based plastics can be degraded in 36 days, WG-soy protein composites degrade to 95% in 30 days, and cotton fibres degrade to 15 to 28% in 90 days, while polypropylene-natural fibre composites degrade only 10% in 100 days (Park *et al.* 2000; Domenek *et al.* 2004; Li *et al.* 2010; Chattopadhyay *et al.* 2011).

In the present investigation, the possibility of producing WG-hemp-based "green" high-quality composites was evaluated. A randomly oriented hemp fibre mat with longer fibre lengths, rather than loose, short, and uni-axially arranged fibres (Wretfors *et al.* 2009; Reddy and Yang 2011b), was used to limit the fibre pull-out effects. Additives and plasticisers are commonly used to improve the quality of bio-based materials (Ullsten *et al.* 2009). In previous studies, plasticisers and water were used to make natural fibre-based composites (Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; Reddy and Yang 2011a); however, in the present study, plasticisers were avoided by using a dry solvent-free method, where the powdered protein matrix infiltrated a preformed mat of hemp fibres to produce wheat protein/hemp composites. This method also maintained fibre length because no high-intensity mixing was needed. Plasticisers, *e.g.*, glycerol, are hydrophilic and absorb a considerable amount of water; they also tend to migrate, leading to poor mechanical properties over time (Reddy and Yang 2011a). Water absorption analysis for WG-based films revealed that for films with 25% and 40% glycerol content, water absorption was 47% and 73%, respectively (Gällstedt *et al.* 2004). Furthermore, in wheat gluten hemp composite, increasing the glycerol content from 20 to 30% was found to decrease the E-modulus from 35 to 10 MPa and increase the extensibility (Kunanopparat *et al.* 2008a). Tensile properties, protein polymerisation, protein type, and biodegradability were investigated for hemp mat-reinforced WG composites produced in the present study. An additional purpose of this study was to increase the understanding of protein polymerisation behaviour, biodegradability, and impact on mechanical properties when various types of wheat gluten proteins, gliadin, and glutenin were used in contact with natural hemp fibres. Furthermore, this study also fills a gap for the production and testing of glutenin- and gliadin-based fibre-reinforced composites, which have not been previously produced.

EXPERIMENTAL

Materials

Wheat gluten was supplied by Lantmännen Reppe AB, Lidköping, Sweden. It had a content of 77.7% gluten protein, 5.8% starch, 6.9% moisture, and 1.2% fats (according to the supplier). The hemp fibre mat (1 cm thick), an industrial product used as landscape mulch, was supplied by Hemcore, United Kingdom. The length of the fibres in the fibre mat was measured manually and showed an average length of 14 mm (2653 fibres, lengths ranging from <1 mm to 135 mm).

Methods

Extraction of wheat gluten proteins

Each of the wheat gluten proteins, gliadin and glutenin, were extracted following the method described in Blomfeldt *et al.* (2012). A quantity of 16 g of WG powder was slowly dispersed in 200 mL of 70% ethanol and stirred constantly to avoid clump formation in the mixture. After mixing the WG powder with ethanol, the solution was placed on a shaker, IKA-KS 500 (IKA, Germany) for 30 min at 300 rpm, and thereafter centrifuged for 10 min at 26,413 g (12000 rpm) in a Sorvall RC 6+ centrifuge (Thermo Scientific, USA). The solution of gliadin in ethanol was decanted and processed in a rotary evaporator (Buchi, Switzerland) at 75 ± 5 °C under vacuum to precipitate the gliadin. Both fractions were lyophilised and ground into powder using an IKA A10 grinder (IKA, Germany) with 2 pulses of 10 s each. The powder size, measured through SEM micrographs (not shown) of WG and glutenin was 2 to 100 µm and that of gliadin was 2 to 10 µm. The powders obtained can be seen as protein powders enriched in gliadin and glutenin; however, they will be referred to as gliadin and glutenin throughout this manuscript. The extraction process was carried out repeatedly and the enriched fractions were pooled until sufficient gliadin and glutenin were obtained for making the composites.

Sample preparation for compression moulding

The dimension of the hemp fibre mats used for sample preparation was obtained through cutting pieces of the mat (5 × 10-cm^2) which were placed at the bottom of a 5 x 10-cm^2 tray. The powder of the WG, gliadin, and glutenin components was poured onto the surface of a hemp fibre mat and shaken for 1 min at 2000 rpm with an IKA-VIBRAX

VXR (IKA, Germany) to allow the protein powder to infiltrate the empty spaces between the fibres prior to compression moulding. After the samples had been produced by compression moulding, the nitrogen contents of the samples and starting materials were measured using the Dumas method, as described in Newson *et al.* (2013); the nitrogen content was used to calculate the ratio of protein to hemp in the samples by the rule of mixtures using the known nitrogen content of the components.

Compression moulding

Based on previous experiments, three different temperatures, *i.e.*, 110, 120, and 130 °C, were selected for compression moulding (Gällstedt *et al.* 2004; Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; Reddy and Yang 2011a). A pressing time of 15 min was applied, and the pressure was kept constant at 400 bars for all samples. The protein-infused hemp mats were placed between polyethylene terephthalate films and two aluminium plates without using a frame around the samples and then placed into the hot compression moulding machine (Polystat 400 s, Servitech, Germany). After compression moulding, the samples were removed from the hot plates and kept between two unheated aluminium plates until the samples reached room temperature.

SE-HPLC Analysis

To obtain a fine powder of the pressed composites, a common steel file (double cut, coarseness-second, Bahco, Portugal) was used. To extract the proteins for SE-HPLC analysis, the method of Gällstedt *et al.* (2004) with modifications according to Blomfeldt *et al.* (2012) was adopted. For each sample, 16.5 mg (± 0.05 mg) was weighed in 1.5-mL Eppendorf tubes and 1.4 mL of buffer solution (0.5% sodium dodecyl sulphate (SDS), 0.05M NaH_2PO_4, pH 6.9) was added. The samples were vortexed for 10 s in a Whirli VIB 2 (Labassco, Sweden) at maximum speed. The samples were further shaken for 5 min at 2000 rpm (IKA-VIBRAX VXR, Germany). After shaking the samples were centrifuged for 30 min at 16,000 RCF (Legend Micro 17, Sorvall, Germany) and the supernatant was directly decanted to HPLC vials (1Ex).

A second extraction (2Ex) was performed from the residuals of the first extraction by sonication (Sanyo Soniprep 150 Ultrasonic Disintegrator, Tamro, United Kingdom). A total of 1.4 mL of the buffer solution was added to the pellet from the first extraction. The sample was sonicated for 30 s at an indicated amplitude of 5 µm and thereafter centrifuged, after which the supernatant was decanted and the sample was placed in the HPLC.

The third extraction (3Ex) of the sample was performed on the pellet from the second extraction; a first sonication of the sample was applied for 30 s, and thereafter it was allowed to cool at room temperature and sonicated again for 60 s. The sample was then centrifuged, the supernatant was decanted, and the sample was placed in HPLC for analyses. All extractions were performed in triplicate.

All collected samples were analysed in a Waters 2690 Separation Module with a Waters 996 Photodiode Array Detector (Waters, USA) based on the method developed by Johansson *et al.* (2001) with modifications according to Gällstedt *et al.* (2004) and Blomfeldt *et al.* (2011). For the analysis, 20 µL of the sample was injected into an SE-HPLC column (Biosep-SEC-S 4000, Phenomenex, USA) with an isocratic flow of 0.2 mL/min (50% acetonitrile, 0.1% TFA; 50% H2O, 0.1% TFA). The 3D chromatograms were extracted at 210 nm (Empower Pro, Waters, USA), integrated, and divided according to the time of elution into two groups depending on the size of the molecules; large polymeric proteins (LPP) - 7.5 to 13 min, and small polymeric and monomeric proteins (SPMP) - 13 to 30 min (Fig. 1). Corresponding figures of raw gluten powder can be seen in Johansson *et al.* (2001) and Nordqvist *et al.* (2013). The integrated data was normalised for initial protein content to compensate for differences in the protein concentration of each individual sample.

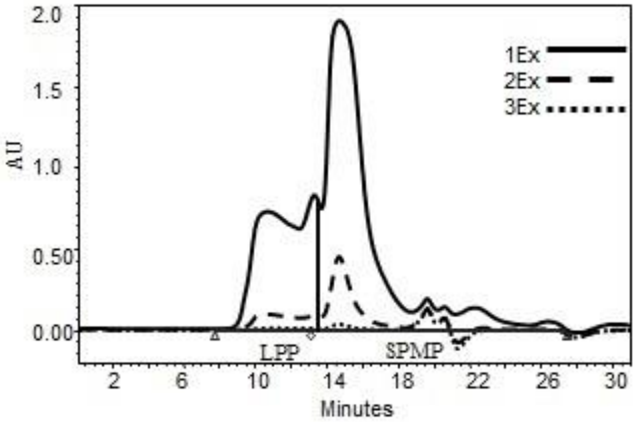

Fig. 1. A representative chromatogram showing extraction and separation of the proteins in compression moulded samples during the first (1Ex), second (2Ex), and third (3Ex) extractions.

Tensile testing

The compression-moulded sheets were laser cut into dumbbell-shaped (ISO 37 type 3) tensile samples. The sample thickness was measured at five different points using a Mitutoyo IDC 112B indicator with a dial gage stand and averaged. Before testing, the samples were conditioned in a climate-controlled room for 48 h at 23 °C at 50% relative humidity. The samples were then tested under the same conditions using a 30-mm clamp separation, crosshead speed of 10 mm/min, and 1-kN load cell. Crosshead displacement was taken to be as elongation of the material similarly as described in Newson *et al.* (2014). The tensile tester used was an Instron 5566 universal test machine using Bluehill software (Instron AB, Danderyd, Sweden) (ASTM-Standard-D638-08 1998). A minimum of 10 samples were tested for each specimen.

Scanning electron microscopy

The morphology of hemp fibre-reinforced WG, glutenin, and gliadin, as well as raw hemp fibres, was carried out using a scanning electron microscope (SEM) (LEO 435VP, Cambridge, UK) equipped with a secondary electron detector with an acceleration voltage of 10 kV. Particular emphasis was directed to observe fibre bonding and fracture surfaces in these samples. Representative samples were selected from the set of tensile tested samples. The samples were coated by sputtering with an Au/Pd 3:2 coating (JFC-1100, JEOL; Tokyo, Japan) before SEM analysis.

Biodegradation evaluation

Evaluation of the biodegradability of the hemp fibre-reinforced WG, gliadin, and glutenin composites was preformed following a procedure similar to ASTM standard D5988-03 (2003) with modifications according to Li *et al.* (2010), wherein the CO_2 release was monitored for 90 days (ASTM D5988-03 2003). However, the samples were kept in soil for an additional 90 days for visual inspection. Soil for the experiment was obtained from an organically grown field situated near the Swedish University of Agricultural Sciences, Alnarp (N55.661303, E13.077222). The soil was passed through a 2-mm sieve and stored at 4 °C for one week before the start of the experiment; thereafter, the pH value and moisture content of the soil were calculated. Two hundred grams of soil was placed in 3 x 6-cm rectangular transparent plastic boxes, and 10 mL of 1 M ammonium phosphate solution was added as a nitrogen source for the microorganisms. The soil containers, together with two beakers (50- and 100-mL capacity), one containing 50 mL of water and the other with 20 mL of 0.5 M KOH, were placed in airtight transparent boxes (4-L) at room temperature (20 ± 4 °C) under darkness. Two-gram samples of WG, glutenin, and gliadin reinforced with hemp fibres were placed in the soil containers and covered with soil. Three replicates of each sample, together with three technical controls, three positive controls, and three soil controls, were used. The CO_2 that evolved during the degradation process was absorbed by 0.5 M KOH placed in the airtight boxes and titrated against 0.25 M HCl at predetermined time intervals. The boxes were kept open for 30 to 60 min while the titration process was carried out for the exchange of fresh air. After the titration process was completed, the KOH beakers were refilled and replaced.

RESULTS AND DISCUSSION

Plasticiser/Solvent-Free Sample Preparation Method

A plasticiser/solvent-free sample preparation method was developed to avoid the use of plasticisers, as well as drying, grinding, and mechanical mixing steps. Drying, grinding, and mixing of fibres require additional energy. In addition to this step, the fibres are damaged during mixing, leading to poor distribution of the fibres in the matrix (Wretfors *et al.* 2009; 2010). Wretfors *et al.* (2009) showed that the presence of longer hemp (length more than 2 mm) fibres leads to poor fibre distribution in the matrix when these are mixed together with a WG/glycerol mixture. A uni-directional fibre alignment has also been used to reinforce WG and soy protein matrices with jute fibres; however, this results in the need for large amounts of water (plasticiser), which in turn requires increasing compression moulding temperatures (Reddy and Yang 2011a,b).

The sample preparation method used in the present study allows the use of a powdered matrix without any plasticiser, solvent, or even water. The advantage of the method is that the fibres are not damaged, as is the case when grinding or mixing is used. Another advantage is the reduced energy consumption during processing. The loss of powder from the fibre mat during handling explains the different WG/hemp fibre ratios obtained (Table 1). The problem of the loss of matrix powder during handling aside, this method promises less energy use and plasticiser-free composites with relatively good tensile properties. The uneven thickness of the hemp mat before pressing also resulted in uneven filling of protein, yielding areas with different mechanical properties; however the thickness of the samples did not show large variations within the samples after pressing (Table 1). Despite the uneven filling of protein, the method yielded dense sheets of WG, glutenin, and gliadin-hemp fibre composites with good morphological characteristics, *e.g.*, esthetical pleasantness, glossy and smooth surface, visible natural fibres, and no air pockets (Fig. 2). The problem with uneven hemp mats might be solved by carefully controlling the mat thickness on an industrial scale, which might result in an even better composite material than the one reported in the present paper.

Table 1. Protein to Hemp Fibre Ratio (wt.%) and Thickness (in parenthesis) of Samples Pressed at Various Temperatures

Temperature °C	WG/Hemp ratio (%)	Glutenin/Hemp ratio (%)	Gliadin/Hemp ratio (%)
110 °C	56/44 (1.52±0.03)	56/44 (1.71±0.03)	62/38 (1.08±0.02)
120 °C	70/30 (1.41±0.02)	48/52 (1.62±0.03)	38/62 (1.23±0.03)
130 °C	63/37 (1.41±0.01)	64/36 (1.44±0.07)	52/48 (1.11±0.02)

Fig. 2. A representative image showing the morphological characters of the hemp fibre-reinforced wheat gluten samples

Effect of Pressing Temperature on the Extraction and Molecular Weight of Proteins

For all the samples, a general decrease was observed in the extractability of proteins with an increase in the compression moulding temperature in the applied temperature range (110 to 130 °C), with the largest decrease seen in the gliadin samples (Figs. 3a, b, and c). The decrease in the extractability of LPP and SPMP during the second and third extractions (using sonication) suggests a higher degree of aggregation between the proteins at higher temperatures for all samples (Figs. 3a, c). The extraction of SDS-insoluble proteins by sonication is a result of the breaking of disulphide cross-links between the protein molecules (Domenek *et al.* 2003; Pommet *et al.* 2004; Kunanopparat *et al.* 2008a; Wretfors *et al.* 2010), while weaker bonds (e.g. hydrogen bonds) and other weak protein interactions are broken already by the SDS-treatment (Kuktaite *et al.* 2004). The total extracted amount of LPP and SPMP decreased with increasing temperature, indicating that the smaller proteins also take part in the cross-linking of the protein matrix (Figs. 3a, b, and c).

At 110 °C and 120 °C, a considerable decrease in protein extractability was observed during the 2nd and 3rd extraction in gliadin-hemp samples compared to gliadin samples (Fig. 3c). Thus, one might speculate that presence of hemp fibres in the samples contributed to polymerization of the gliadins. Previous studies have shown that the plasticiser glycerol act as a chemical chaperon for gliadins at compression moulding. Thus, it might be possible that the hemp fibres contribute with some kind of chemical additives or other chemical properties that also may induce a higher polymerization rate of the gliadins.

In general and in accordance with previous findings (Kunanopparat *et al.* 2008b; Wretfors *et al.* 2009), the presence of hemp fibre in the WG protein samples did not seem to induce large variations in the aggregation of WG proteins. However, in this study, slightly higher protein extractability was observed in hemp fibre-reinforced WG and glutenin composites at 120 °C. Hemp fibres are mostly composed of cellulose, hemicellulose, and lignin, with small amounts of pectin and waxy substances which are considered surface impurities (Mwaikambo and Ansell 2002). The chemical composition of the hemp fibres might be the background for influences seen in protein polymerization at different temperatures. The loss of solubility due to protein unfolding, cross-linking, and aggregation is known to occur at a greater extent at higher temperatures, thereby leading to moulding temperature-dependent mechanical properties (Domenek *et al.* 2002; Morel *et al.* 2002; Kunanopparat *et al.* 2008b; Wretfors *et al.* 2010).

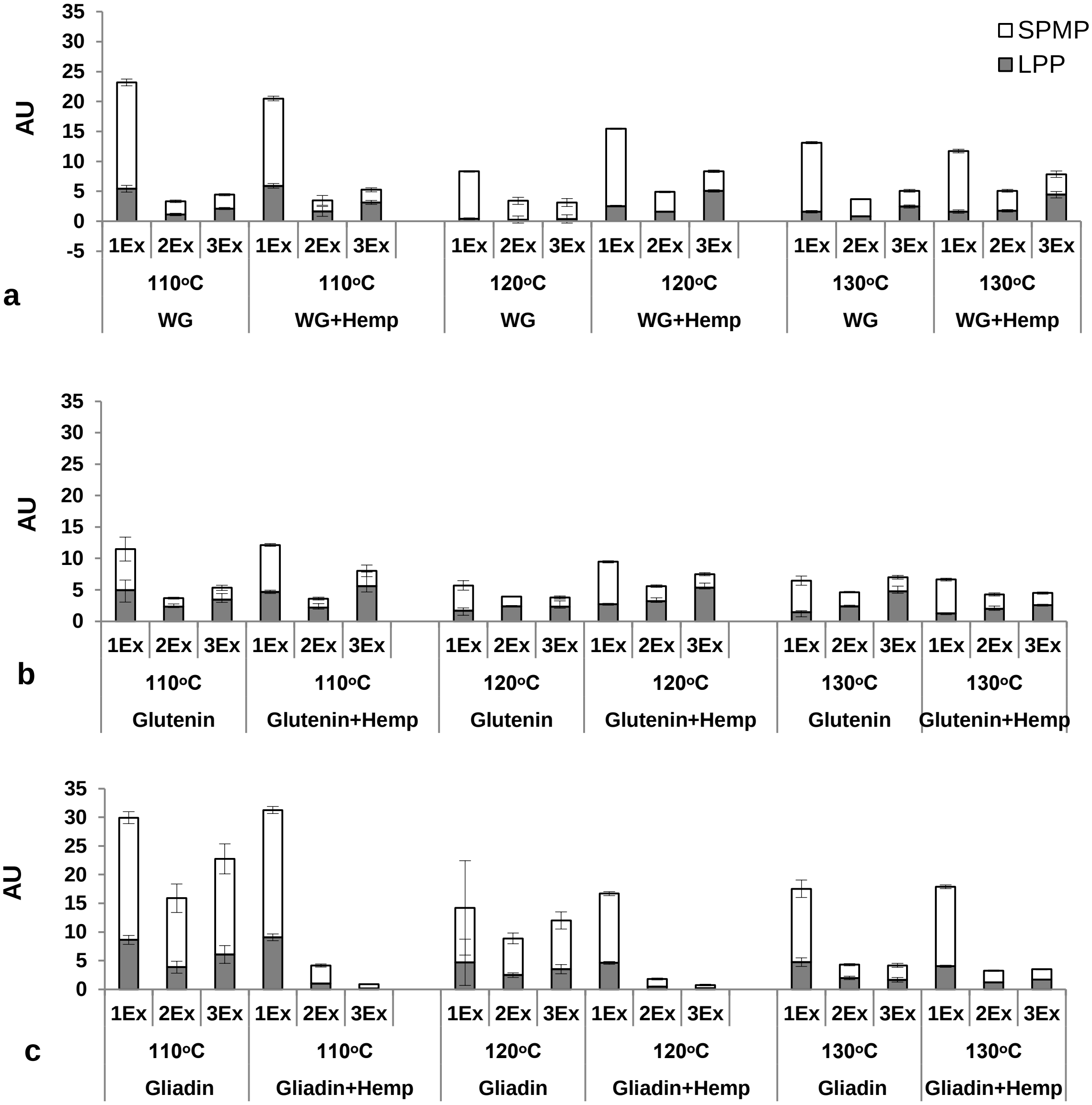

Fig. 3. Effect of temperature on the polymerisation of small polymeric and monomeric proteins (SPMP) and large polymeric proteins (LPP) in (a) compression moulded WG and hemp fibre-reinforced WG composites, (b) compression moulded glutenin and hemp fibre-reinforced glutenin composites, and (c) compression moulded gliadin and hemp fibre-reinforced gliadin composites. Bars represent the standard deviation.

Tensile Properties

Representative tensile behaviour of the compression-moulded hemp fibre-reinforced WG-, glutenin-, and gliadin-based composites are shown in Fig 4. The protein-to-hemp ratio differed in the samples, as a limiting factor of the sample preparation method; however, no clear correlation between the protein/hemp ratio and the tensile data was observed (Table 1, Fig. 5). Furthermore, the standard deviation in the tensile properties did not show large variations (Fig. 5). These results do not correspond with others (Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; 2010), showing that fibre ratio largely influences tensile properties. One reason for the diverging results might be that the stiffness of the fibres and the matrix in the present study probably do not differ substantially. A comparison between the samples without hemp and those with hemp fibres produced at 130 °C revealed that the hemp contributed primarily to an increase in E-modulus (Fig. 5a) due to the fact that hemp fibres have a higher E-modulus than the protein matrix (Kunanopparat *et al.* 2008b). Previous studies have shown that addition of hemp fibres to a wheat gluten matrix increased both the E-modulus and the maximum tensile stress (Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; 2010).

The temperature during the compression-moulding was found to especially influence the maximum stress (Fig. 4c). In general, the gliadin samples were stronger and stiffer than the glutenin samples (Figs. 5a, c). Gliadin samples also showed higher E modulus than the other samples, especially at high processing temperatures. These results also correspond well to other results showing interesting tensile properties especially of gliadin based materials (*e.g.* Blomfeldt *et al.* 2012; Rasheed *et al.* 2014; unpublished results). One reason suggested for the better properties of gliadin- compared to glutenin-based materials is that the opportunities to fully denature and depolymerize the proteins during processing so that they can re-polymerize again are much higher in gliadins than in glutenins (Rasheed *et al.* 2014). In previous studies, compression moulding temperatures between 75 and 130 °C were used to produce wheat gluten plastic material with a similar matrix as that seen in the present study, and the modulus and maximum stress properties are in most cases significantly improved when pressing temperature increases from 120 to 130 °C (Gällstedt *et al.* 2004; Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009; 2010). A suitable temperature that has a positive influence on maximum stress and modulus of the material is known to induce cross-linking and intermolecular covalent bonding between proteins (Gällstedt *et al.* 2004; Reddy and Yang 2011a).

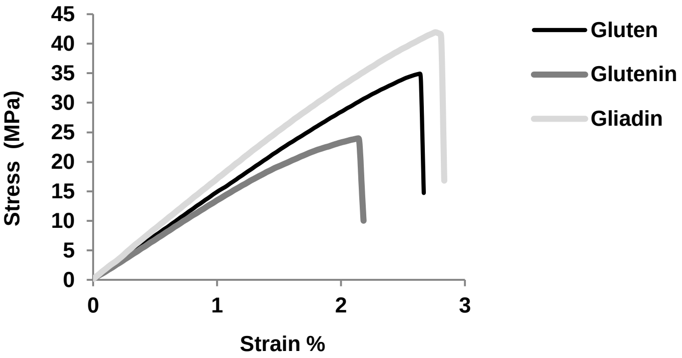

Fig. 4. Representative tensile behaviour of hemp fibre-reinforced WG, glutenin and gliadin composites (130 °C).

A comparison of the tensile properties of WG/hemp fibre-based composites produced in previous studies and the present one showed that the latter composites had a higher E-modulus and maximum stress and a lower extensibility (Kunanopparat *et al.* 2008a; Wretfors *et al.* 2009). Possible reasons for the higher E-modulus and maximum stress values in the present study are the use of a mat with randomly oriented fibres and the method used to make the composite without damaging the fibre surface or decreasing the length. Furthermore, no plasticiser and mixing were used in the present study, contributing to differences in manufacturing methods, making results difficult to compare. Wheat gluten reinforced with 20% (wt.) hemp fibres and 30% glycerol, compression-moulded at 130 °C, showed a lower modulus and strength and a higher extensibility (Wretfors *et al.* 2009) than the present composites with the hemp mat. The reasons for these differences may be the higher hemp content and lack of plasticiser in the WG-hemp composites. The higher stiffness and strength found in a previous study on jute fibre composites (Reddy and Yang 2011b) may be due to low levels of plasticizer (water) in the tested state, the uniaxial alignment of the jute fibres, and the fact that the composite was tensile tested in the fibre direction.

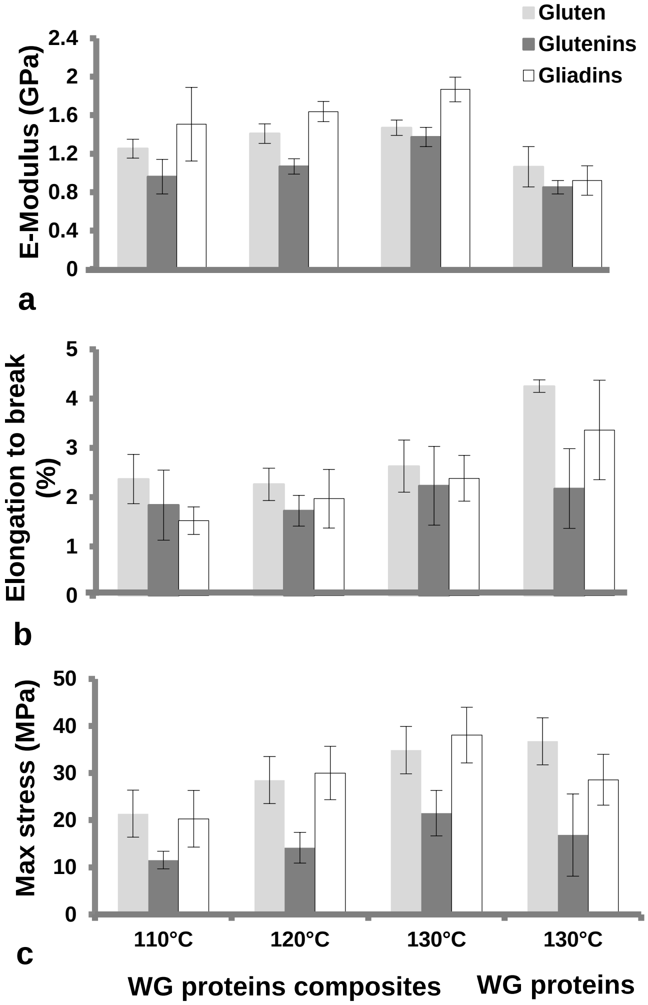

Fig. 5. Effect of temperature and protein type on (a) E-Modulus, (b) elongation at break and (c) maximum stress of the hemp fibre-reinforced proteins (WG, glutenin, and gliadin separately) compared with protein samples compression moulded without hemp fibres. Bars represent the standard deviation. For fibre content in each of the samples, (Table 1).

Tensile Fracture Surface Analysis

Representative micrographs from the SEM analysis are shown in Fig. 6. The fracture surface analysis of the tensile tested hemp fibre-reinforced WG, gliadin, and glutenin composites showed some fibres being broken at fracture, although the fibres were mostly pulled out from the protein matrix, leaving “clean” holes (Fig. 6c). The fibres that were pulled out were similar in appearance to the raw hemp fibres shown in Figs. 6a and 6b. Hemp fibres contain hydroxyl groups, which are readily available for hydrogen bonding with the matrix; however, the compounds present on the surface of the fibres tend to act like a barrier, making hydroxyl groups unavailable for reaction (Mwaikambo and Ansell 2002). As observed in Fig. 6, this evidently reduces the adhesion between the fibre and the protein matrix and consequently reduces the reinforcing effect of the fibres. An option to improve the fibre bonding to the matrix is to use various types of chemical treatments of the fibres before making the samples. One such type of chemical treatment that has been shown to improve the fibre-to-matrix reaction interface, ultimately resulting in an improvement in the mechanical properties, is treatment with NaOH solution before processing (Mwaikambo and Ansell 2002). However, in the present study, we aimed at the production of sustainable composites and thereby preferred not to use chemical additives to avoid the environmental impact of disposal of alkali solutions from the alkali treatment of fibres. Instead, we used a randomly oriented fibre mat to try to connect fibres to each other through mechanical interlocking to avoid pull-out of fibres. The used methodology, including the randomly oriented fibre mat, must be seen as successful due to the fact that tensile properties were substantially improved, although, as expected, pull-out was seen at fracture break.

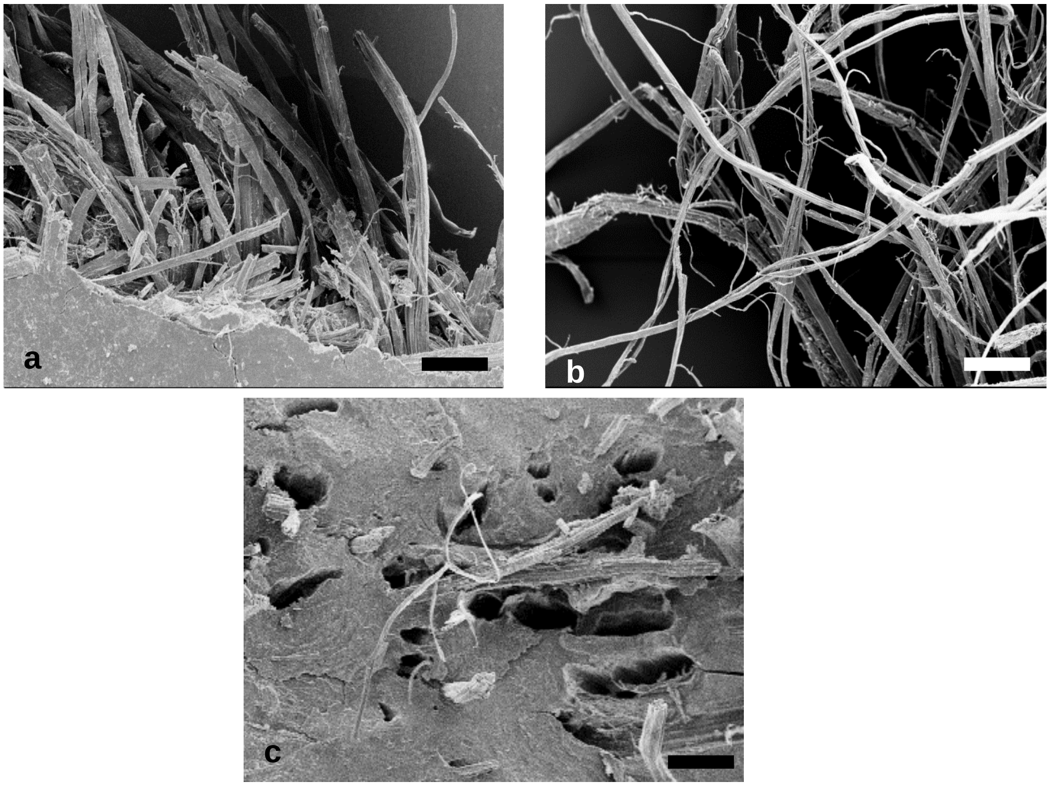

Fig. 6. SEM micrographs showing fibre pull-out in (a) hemp fibre-reinforced wheat gluten, compression moulded at 130 °C , (b) raw hemp fibres, and (c) clean holes after fibre pull-out (bars correspond to 100 µm for a, b, and c)

The powder infiltration technique allows long hemp fibres to be incorporated, increasing the probability of mechanical interlocking of features on the fibre surface to improve fibre/matrix load transfer, resulting in fibre breakage before pull out. All previous studies have shown a pull-out effect of hemp fibre in wheat gluten-hemp and soy protein-hemp composite materials (Mohanty *et al.* 2005; Wretfors *et al.* 2009). In the present study, no signs of damage to proteins or adhesive were seen on the surface of the fibres that were pulled out during the tensile testing process, as has been previously reported (Wretfors *et al.* 2009). One possible reason for fibre pull out seen in the present study might be microscopic voids around the surface of interlocking fibres in the pressed samples, as these areas could be too small for adequate powder infiltration. These voids may be causing a clean pull out of fibres together with poor fibre-matrix interaction. It would have been interesting to calculate the theoretical and actual density of the fibre mat, matrix, and prepared composites in order to better understand the composite behaviour and eventual presence of voids. However, such a calculation is easily carried out on traditional composites, while biobased materials complicate the procedure, not least because theoretical densities on hemp fibers and protein matirices are lacking and also hemp fibers are varying in their size, thickness, length, *etc*.

Biodegradation of the Composites

The percentage of carbon converted to CO_2 by the activity of microorganisms was determined as a measure of biodegradation. The total calculated amount of CO_2 after 90 days (ASTM D5988-03 2003) is shown in Fig. 7. The total rate of biodegradation differed for the investigated samples, with higher rates for WG and gliadin compared to glutenin composites. The extent of degradation was 30 to 40% after 90 days (Fig. 7). The samples were also monitored visually; after 180 days, there were no physical signs of materials left in the soil (Figs. 8a and 8b), thereby not allowing weighing of the samples and also indicating full degradability, as has also been found after 50 days for plasticised wheat gluten samples subjected to farmland soil (Domenek *et al.* 2004). Biodegradation analysis of natural fibre-reinforced polypropylene composites showed a slower degradation rate than the composites in the present study, with a degree of biodegradation of less than 20% over a 180-day period (Chattopadhyay *et al.* 2011). In another study, a simple weight loss calculation for soy protein and WG films subjected to farm-land exposure revealed that the materials were 50% degraded in 10 days, with a 95% weight loss observed after 30 days (Park *et al.* 2000). Thus, the present study showed a somewhat slower degradation of the hemp fibre-reinforced gluten samples compared to what has been found in other bio-based materials, perhaps due to the lack of hygroscopic plasticisers and high cross-linking density of the proteins. Other factors that might influence the biodegradation rate are the fact that hemp fibres degrade more slowly than the protein matrix. Comparison of influences of various types of fibres, protein matrices, and plasticiser to the biodegradation rate would be interesting although, at present, very little information on biodegradation kinetics of biobased materials is available in the literature. In the present study, the rate of biodegradation was higher than that of synthetic matrix-based natural fibre composites (Chattopadhyay *et al.* 2011). Visual inspection showed that the proteins in the composite clearly degraded faster than the hemp fibres, with the composites losing cohesion due to matrix degradation while the relatively low fibre degradation rate contributed to the overall lower biodegradation rate. This lack of cohesion precluded monitoring the weight loss of the samples during degradation.

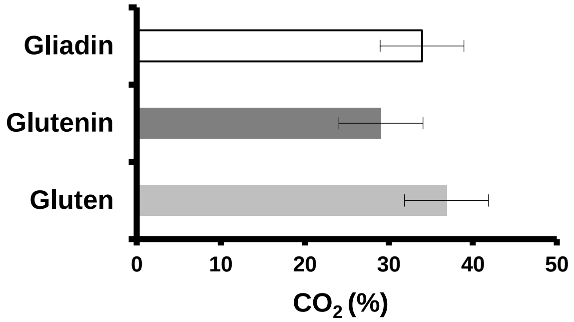

Fig. 7. The amount of CO_2 released from compression moulded hemp fibre-reinforced WG, gliadin, and glutenin protein samples after biodegradation in soil for 90 days (bars correspond to 100 µm for a, b, and c)

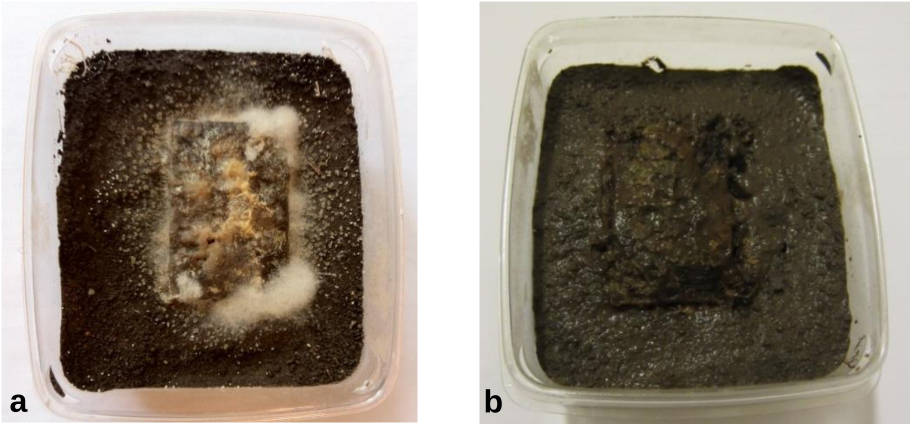

Fig. 8. Example of biodegradation of the hemp fibre-reinforced WG composite (WG/Hemp ratio 70/30 produced at 120 °C) after (a) 45 and (b) 180 days. The sample in the picture was used for visual analysis (samples for data collection were buried in the soil).

CONCLUSIONS

1. The present study proposed a method for the formation of wheat gluten (WG) and hemp fibre-based composites that avoids the use of additives, plasticisers, or processing aids, which contributed to a low chemical load on the environment and decreased production and energy costs during processing.
2. Furthermore, the proposed method does not involve the use of energy-consuming processes such as mixing, grinding, and drying, which can also damage the fibres and hence affect the final mechanical properties.
3. This study also indicates that it is possible to use a higher content of hemp fibre (up to 62 wt.%) and longer fibre lengths without negatively affecting the cross-linking of the protein matrix. Despite the long fibre lengths and high fibre content, the materials produced were processable *via* compression moulding.
4. The biocomposites from WG and hemp were shown to be fully biodegradable, although with a longer degradation time than has been reported for other fully biodegradable bio-based materials. Thus, the composite materials studied here proved to have environmentally "green" potential and good mechanical properties.

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