Protein Hydrolysates from Biogenic Waste as an Ecological Flame Retarder and Binder for Fiberboards

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ABSTRACT: The increasing demand for sustainable building materials requires alternative flame retarders, which have superior sustainability to those previously used. In this respect, we present our initial results with protein hydrolysates made from poultry-feather waste for the preparation of flame-retardant fiberboards. Impregnated wood fibers show a significantly decreased decomposition rate in the region between 300 and 450 °C, as measured by thermogravimetric analysis. Final combustion of the impregnated fibers is shifted up by 50 °C to the interval 450–500 °C and occurs stepwise rather than instantaneously as for untreated wood. At a total protein content of approx. 10 wt %, plates produced in the “wet” process are self-extinguishing and show very little subsequent smoldering. In three-point bending tests, these fiberboard prototypes were able to withstand stresses of up to 15 N/mm², the threshold required by DIN EN 622 for commercial, formaldehyde-bound MBH fiberboards. This indicates that the upcycled protein hydrolysates not only have an impressive flame-retarding effect but also can be used as a fully sustainable binder for a new generation of ecological fiberboards. As these boards are based solely on natural materials, they can be shredded and composted at the end of their life cycle.

INTRODUCTION

The recent rise in the number of large and severe fires such as in the Grenfell Tower (London 2017), the entertainment centre Simnja wischnja (Kemerowo 2018), the Brazil National Museum (Rio de Janeiro 2018), Notre Dame (Paris 2019), and the Abbco Tower (Scharscha, UAE, 2020) has brought the importance of flame retarders back into public awareness. Although there is a large number of trusted and reliable substances available on the market, the re-evaluation of toxicological aspects, for example, as brought forward by the regulation (EG) no. 1907/2006 of the European Union known as REACh, and increased environmental awareness has led to a number of proven retarders such as antimony or brominated compounds being banned or viewed critically. For example, the previously common flame retarder hexabromocyclododecane (HBCE) was classified as a persistent, bioaccumulative, and toxic substance and restricted by means of a sales ban. Other substances such as antimony(III) oxide are listed as possible carcinogens by the International Agency for Research on Cancer and are still under investigation within the REACh process. Therefore, environmentally friendly and “green” fire retarders are the subject of a number of current research efforts. In addition, the quest for increased sustainability has led to wood becoming increasingly popular again, not only for interior fittings but also as a building material for multistorey buildings. Bringing these two recent trends together in order to provide large quantities of sustainable, 100% biological, and nontoxic materials for fire-protected wood buildings requires novel approaches.

There have been some attempts to use proteins as flame retarders. Of these, casein is very promising as it naturally contains high amounts of phosphorus; however, casein intervenes with the food chain and is, therefore, unsuitable for large-scale technical applications. Another interesting protein is hydrophobin, which consists of 100 amino acids with 8 cysteine units and is similarly found to reduce the burning rate of cotton. The composition of hydrophobin is similar to that of the very abundant protein keratin, which is found in beaks, horns, hoofs, hair, and feathers. However, these are usually considered as waste, as they have very few value-added uses. This is despite the fact that keratin has some remarkable thermal properties such as a high decomposition temperature. In this context, we have also recently shown the beneficial effects of shredded poultry feathers on the thermal stability of biobased, polycondensation-type thermoset composites. Moreover, keratin does not burn—hence, keratin...
Viscous phase. After decanting, the solution is acidified and centrifuged to obtain a clear, yellow protein solution and a room temperature for 24 h. The resulting turbid solution was washed with distilled water, and finally dried by lyophilization.

In a typical experiment, 10 g of shredded feathers was added to the reaction mixture and stirred for 3 h at 50 °C. The pH is checked and readjusted to pH 12 every 15 min during the reaction.

Wood Impregnation. The impregnation solutions were prepared by dissolving 70 mg/mL of the dried protein hydrolysate in 0.2 M NaOH and mixed with wood fibers in ratios of 0.5, 1, and 1.5 mL/g for 5 min. The impregnated fibers were finally dried at 50 °C to constant weight.

**MATERIALS AND METHODS**

Goose feathers (Figure S1A) were provided by Treude & Metz GmbH, Bad Laasphe, Germany, as a random mixture of tail and chest feathers as well as down. The mixture was cut to a uniform size on a Retsch SM cutting mill fitted with a 200 μm sieve (Figure S1B). Dust-free beech wood fibers with a size of 0.5–1 mm (RG HB 500/1000) were obtained from J. Rettenmaier & Söhne GmbH + Co KG, Germany (Figure S1B). Analytical-grade NaOH and Na₂P₂O₇ (STMP) were from VWR, Germany, and used as received.

**Alkaline Hydrolysis of Feathers and Phosphorylation.**

In a typical experiment, 10 g of shredded feathers was suspended in 100 mL of 1 M NaOH solution and shaken at room temperature for 24 h. The resulting turbid solution was centrifuged to obtain a clear, yellow protein solution and a viscous phase. After decanting, the solution is acidified with 1 M HCl to pH = 5 and the precipitated protein is filtered, washed with distilled water, and finally dried by lyophilization. Phosphorylation of the protein hydrolysate followed a published procedure. For that, 2 g of the dried keratin hydrolysate was mixed with 50 mL of water and the pH was adjusted to 12 with 2 M NaOH. A total of 1.5 g of STMP was added to the reaction mixture and stirred for 3 h at 50 °C. The pH is checked and readjusted to pH 12 every 15 min during the reaction.

**Amino Acid Analysis.**

A mixture of 20 mg of the dry sample, 5 mL of 6 N hydrochloric acid, and a few drops of 0.1% aqueous phenol solution (antioxidant) was heated at 110 °C for 24 h. After cooling, the sample was transferred quantitatively into a 10 mL volumetric flask. The excess of hydrochloric acid was removed on a rotatory evaporator at 50 °C. The residue was washed several times with distilled water and finally dried in a stream of dry nitrogen before being dissolved in a lithium acetate buffer at pH 2.2. The amino acid analysis was performed by ion exchange chromatography after column derivatization with ninhydrin. Because of the acidic conditions in the first step, glutamine and asparagine cannot be detected as they are hydrolyzed to glutamic and aspartic acid; tryptophane is completely destroyed in the process.

**ATR–FTIR Spectroscopy.**

Infrared IR spectra were recorded using a PerkinElmer Spectrum Two UATR FTIR spectrometer equipped with a diamond ATR (attenuated total reflection) window. All spectra were recorded in the spectral range of 4000–400 cm⁻¹ with 12 scans at a spectral resolution of 4 cm⁻¹. Before each measurement, the diamond ATR crystal was cleaned with isopropanol.

**Thermogravimetric Analyses.**

Measurements were performed on a PerkinElmer TGA 4000 instrument heating from 30 to 850 °C at a rate of 10 K/min under a continuous nitrogen or oxygen flow rate of 20 mL/min. Samples were stored in a standardized climate (RH 55%; T = 21 °C) prior to the measurements. The sample weight was set to 100% at 150 °C, where residual water was completely evaporated, to allow better comparability between samples.

**Horizontally Burning and Smouldering Tests.**

Tests were performed on a horizontal specimen following the UL94 standard. For sample preparation, 1 g of treated or untreated wood fibers was evenly distributed in a rectangular mould of 7 × 1 cm² and pressed at 150 °C and 10 bar/cm² machine pressure for 10 min into approximately 1.2 mm-thick plates. The plates were marked at intervals of 5 mm over a length of 50 mm, mounted horizontally at one end, and lit at the other. The experiments were filmed and evaluated with an image-processing software.
Mechanical Testing. A total of 1.5 g of treated or untreated dry wood fibers was evenly distributed in a rectangular mould of 7 × 1 cm² and the surface was sprayed evenly with distilled water to obtain a moisture content of approx. 15 wt %. The samples were pressed at 150 °C and 10 bar/cm² machine pressure for 10 min into approx. 2 mm-thick plates. The three point bending test was performed on an Instron 5566 universal testing machine with a free length of 35 mm, an initial load of 1 N, and a strain rate of 1 mm/min.

RESULTS AND DISCUSSION

The feathers were received both washed and greasy, but the fat content was not found to have any influence on the hydrolysis as the same results, such as, for example, the time−concentration profile shown in Figure 1A, were obtained with both types. Alkaline hydrolysis of the shredded feathers produces a turbid solution than can be separated by centrifugation into a clear solution, containing soluble protein fragments, and a viscous phase, which consists of still partially cross-linked protein chains. The following work focuses on the clear protein solution, although the viscous phase can also be transformed into value-added products (this is beyond the scope of this article). A typical time−conversion curve for the alkaline hydrolysis of the shredded feathers at room temperature is shown in Figure 1A. It should be noted that the feathers contain approx. 8% of water under ambient conditions. Thus, the maximum amount of dissolved keratin under the present experimental conditions is 0.092 g/mL. Within 50 h, approx. 80% of the feathers are solubilized, but such extended reaction times also lead to the formation of large amounts of very small and undesired protein fragments, as observed using gel electrophoresis (Figure 1B). In order to obtain longer chains for the following investigations, the hydrolysis is usually stopped after 24 h (approx. 70% solubilization) at the expense of producing more of the viscous phase.

Alkaline hydrolysis of the feathers not only decreases the length of the protein chain but also eliminates the disulphide bridges that cross-link the protein. This improves the solubility and allows higher protein concentrations in the impregnation solution but also causes a change in amino acid composition (Table S1). The most notable change is the overall decrease in cysteine by about 78%, which is most likely caused by β-elimination from cystine in the alkaline environment to form dehydroalanine and a persulphide (Figure 2).15–17 Both persulphide and dehydroalanine are rather reactive and can undergo subsequent reactions, for example, with other amino acids, which may account for some of the differences in the amino composition of the feathers and the hydrolysate. In addition, some of the more water-soluble amino acids might be lost during work-up of the hydrolysate so that more hydrophobic ones such as leucine (+14%) and proline (+18%) might be overestimated. The observed decrease in sulfur-containing amino acids is beneficial for the application as a flame retarder as the amount of harmful sulfur oxides formed in the case of a fire is decreased.

Because phosphate derivatives are known to improve the flame-retardant properties of materials, the protein hydrolysates were reacted with sodium trimetaphosphate (STMP) in alkaline solution following an established procedure.12 STMP is known to react with both serine and lysine, but based on the amino acid analysis in Table S1, serine with a content of 10.8% (as compared to lysine with 0.6%) appears to be the prevalent reaction partner in the present case. Infrared spectra of the product show a new absorption band at 1083 cm⁻¹, which is in accordance with the literature and indicates the presence of phosphate groups.18 However, the amount of serine does not decrease significantly after phosphorylation (Table S1) and the literature is divided on whether or not the phosphate group is

![Figure 2](https://dx.doi.org/10.1021/acsomega.0c03819)

**Figure 2.** β-Elimination cleaves a disulfide bridge into dehydroalanine and a persulfide. The stars denote the continuation of the protein chains.

![Figure 3](https://dx.doi.org/10.1021/acsomega.0c03819)

**Figure 3.** TGA (A) and dTG (B) of feathers (black solid line), beech wood fibers, protein hydrolysate, and phosphorylated hydrolysate under an oxygen atmosphere.
really covalently bound or only physically adsorbed on the protein surface.19

For TGA, an enriched oxygen atmosphere (approx. 35 vol % O2) was used to simulate the conditions during a fire because TGA under a nitrogen atmosphere more resembles pyrolysis. All four materials under investigation show a rather similar decomposition behavior up to a temperature of approx. 300 °C (Figure 3). Beyond 300 °C, the decomposition of beech wood accelerates and between 300 and 330 °C, the residual weight decreases from 75 to 40% of the initial weight. This was previously associated with the decomposition of the hemi-cellulose.20 The beech wood is fully decomposed at 450 °C. For the three keratin-based materials, the mass loss above 300 °C proceeds at a lower rate compared to beech wood (approx. 60% of the initial weight at 330 °C) and both feathers and hydrolysate are only fully decomposed at approx. 600 °C. The thermal behavior of the hydrolysates was found not to depend much on the time of hydrolysis beyond 8 h of the reaction. Shorter times (<8 h) produced materials with slightly faster decomposition (data not shown). The behavior of the phosphorylated hydrolysate does not differ substantially from that of the feathers or the pure hydrolysate in the region up to 500 °C. However, decomposition of the phosphorylated keratin leaves a residual mass of approx. 7%, which is most likely because of the phosphate content and not a sign of an improved thermal stability. Because the additional phosphorylation step reduces the sustainability of the presented approach, the phosphorylated keratin hydrolysate is not considered in the following experiments.

The degradation behavior is similar for all samples up to approx. 300 °C with a mass loss of approx. 27% (Figure 5A). As already shown in Figure 3, the untreated wood then undergoes rapid decomposition and is fully decomposed at 450 °C. The wood sample treated with 0.5 mL/g is rather similar to pure wood; however, increasing the amount of keratin continuously (to 1.0 mL/g and 1.5 mL/g respectively) slows down the degradation rate in the temperature region between 300 and 450 °C and the degradation behavior of the treated wood gradually approaches that of feathers. This is in accordance with previous observations using urea or guanidine carbonate on cotton and was addressed to a change in the reaction energetics from endothermic to slightly exothermic.22 At 450 °C, the wood sample treated with 1.5 mL/g shows a residual weight of approx. 31%, while the untreated wood is fully decomposed. It should be noted that the absolute amount of keratin in this sample is only 10.5 wt % and, yet, the thermal profile matches that of pure feathers in this temperature region. Starting at 455 °C, the samples treated with 1 and 1.5 mL/g begin decomposing, which ends slightly above 500 °C with a residual mass of approximately 3%. However, the final combustion of the sample treated with 1.5 mL/g appears to be stepwise as seen in the dTG curves (Figure 5B) indicating that the remaining protein still interferes with the decomposition mechanism.

The solutions used for the impregnation of wood are prepared by dissolving 70 mg/mL of the isolated hydrolysate in 0.2 M NaOH solution. This concentration was found to be the solubility limit at that pH. Using more concentrated NaOH solutions in order to obtain more concentrated impregnation solutions is possible, but it was considered impracticable in view of potential future applications. Higher concentrations of keratin inevitably increase the solution viscosity, whereas the solutions should have a low viscosity to allow complete impregnation of the wood fibers. Within this study, the impregnation solutions were prepared from the isolated protein hydrolysate in order to achieve standardized conditions, but the crude hydrolyzed mixtures could also be used after adjusting the protein concentrations.

The wood fibers were impregnated with these solutions in ratios of 0.5, 1, and 1.5 mL/g. The latter is about as much as the wood can absorb without appearing wet. The uptake of the keratin solution by the wood fibers proceeds in two steps, an initial, rather quick filling of the wood pores followed by the slower swelling of the wood matrix. ATR−Fourier-transform infrared (FTIR) spectroscopy of the impregnated wood shows the typical bands of beech wood, for example, at about 1030 cm⁻¹, and the amide-I and -II bands of keratin, for example, at about 1630 and 1516 cm⁻¹ (Figure 4).

The treated and thoroughly dried wood fibers were subject to TGA under nitrogen (not shown) to determine the char residue at 850 °C. This correlates with the limiting oxygen index (LOI) that indicates the volume concentration of oxygen needed for combustion to occur.21 Impregnation with the protein solution increases the LOI from 23 (char residue 14%) to 26 (char residue 21%), and this is not due to the sodium content in the impregnation solution, as this constitutes only 0.7% of the total mass. However, TGA under nitrogen resembles pyrolysis more than a fire event, so the analyses were repeated in an enriched (approx. 35 vol %) oxygen atmosphere (Figure 5).

The treated and untreated wood fibers were pressed into 7 × 1 cm² plates to test their flammability and to verify the flame-retarding effects of the keratin hydrolysate indicated in the TGA measurements. For a proof-of-principle experiment, a horizontal setup following the UL 94 standard was chosen to evaluate the burning distance, speed, and time. All plates made from untreated wood burn with a large shining flame and burn down the complete distance of 50 mm. In contrast, the impregnated plates burn with a less bright flame and extinguish after several millimeters (Figure 6).
The results of the flammability tests are summarized in Table 1. Besides the self-extinguishing behavior, the keratin-impregnated plates burn at about half the rate of the untreated ones.

In certain wall constructions such as concealed wood fiber insulations, smouldering is an even greater danger than the actual fire. To evaluate the smouldering properties, the samples were continued to be monitored after being extinguished with a CO₂ extinguisher (untreated plates) or self-extinguishing (treated plates). The untreated samples showed a massive smouldering for up to 5 min during which most of the unburnt sample was consumed. In contrast, the impregnated samples showed only a slight smouldering after self-extinguishing, which did not consume any unburned material and subsided within a minute.

Three point bending tests were used to evaluate the material properties. Because no glue is used to prepare the plates, it was of particular interest to assess the effect of the keratin impregnation on the fiber–fiber adhesion, which directly reflects the strength of the plates. Samples prepared from untreated wood fail at 5 N/mm², while those prepared from keratin-impregnated wood can withstand up to 15 N/mm² (Figure 7). This is exactly the strength prescribed in DIN EN 622 for medium-density fiberboards prepared from the “wet” process (type MBH) with a thickness of <10 mm. The bending modulus of untreated boards is 455 ± 118 N/mm² and of keratin-treated boards is 1398 ± 307 N/mm².

Table 1. Evaluation of the Flammability Tests on Horizontal Plates

| parameter       | untreated (n = 8) | keratin-impregnated (n = 7) | change (%) |
|-----------------|-------------------|----------------------------|------------|
| distance/mm     | 50 ± 0⁰           | 14.1 ± 9.4⁰                | −72        |
| time/s          | 116.5 ± 7.9       | 48.4 ± 27.6                | −59        |
| rate/mm·s⁻¹     | 0.43 ± 0.03       | 0.28 ± 0.07                | −44        |

*Impregnated with 1.5 mL/g of a 70 mg/mL hydrolysate solution in 0.2 M NaOH. The sample burned down the complete distance under investigation. The fire ended with self-extinguishing.

Figure 5. TGA (A) and dTG (B) of untreated wood fibers and keratin-impregnated wood fibers in an oxygen atmosphere.

Figure 6. Horizontal flammability test shown for untreated and keratin-impregnated plates that closely resemble the average values given in Table 1. The distance between the black marks at the bottom edge of the plates is 5 mm.

Figure 7. Evaluation of mechanical parameters of treated and untreated keratin plates.
**CONCLUSIONS**

Protein hydrolysates are efficient flame retarders for wood fibers and fiberboards. The hydrolysates can be made in an eco-friendly process at room temperature using biogenic protein waste as a resource. Data provided by TGA suggest that the protein hydrolysate slows down the hemicellulose decomposition leading to a reduced overall decomposition rate between 300 and 450 °C. For wood-based materials, these changes in the TGA curves could be used as an indicative tool to test for an increased degree of flame retardation. In a flammability test following the UL 94 standard, plates pressed from the treated wood fibers were self-extinguishing and showed significantly reduced burning distances and rates. In a real fire event, flames would spread less quickly across keratin-treated fiberboards providing more time for evacuation or firefighting operations. The protein hydrolysate also significantly improved the smouldering behavior, an important safety feature for wood fiber insulations. In addition, plates pressed from protein-treated wood fibers showed bending strengths similar to commercial fiberboards bound with formaldehyde resins, the latter having the major drawback of being volatile organic compound (VOC) releasers. Added keratin can also be expected to adsorb VOCs, as already shown for wool, and this will be the subject of further studies. The possibility of making flame-retarding binders from biogenic waste creates added value and is a true upcycling that paves the way for a more sustainable future.

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