Ultrasound Assisted Alkaline Pre-treatment Efficiently Solubilises Hemicellulose from Oat Hulls

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Abstract

The establishment of sustainable bioeconomies requires the utilization of new renewable biomaterials. One such material currently seen as a waste product is oat hulls. Oat hulls exhibit a great potential for the production of dietary fibres due to their exceptionally large hemicellulose content (35%). Their recalcitrant structure however requires a suitable pre-treatment method to access and process the hemicellulose. After a screening of various physical, chemical and physico-chemical pre-treatment methods, including autoclaving, ultrasonication, microwave-, deep eutectic solvents-, as well as alkaline treatments, a combined ultrasonication and alkali pre-treatment method was here found to be the most suitable. A factorial design resulted in optimized conditions of 10 min ultrasonication in water, followed by an incubation in 5 M NaOH at 80 °C for 9 h yielding solubilisation of 72% of all hemicellulose in the hulls. The method was shown to efficiently break the ester bonds between ferulic acid and the hemicellulose main chain, contributing to its solubilisation.

Graphic Abstract

Keywords Oat hulls · Ultrasonication · Alkali pre-treatment · Hemicellulose solubilisation

Statement of Novelty

The successful transition of modern economies to sustainable bioeconomies requires the development of suitable methods for the utilization of renewable biomaterials. Oat hulls are currently an underutilized agricultural side stream with interesting chemical properties. Their exceptionally

large hemicellulose content makes them great candidates for the production of health promoting food ingredients, such as dietary fibres. Due to the heavy crosslinking of its lignocellulose components, a suitable pre-treatment method is required. The in this study developed method yields a very high amount of soluble hemicellulose; higher than previously reported for oat hulls or other lignocellulosic material.

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Introduction

With a growing world population, an increase in food production quantities is required. Alongside the production of food, larger amounts of agricultural side streams are generated. To date, those are mainly underutilized and often regarded as waste leading to improper handling increasing the risk for serious environmental pollution [1]. Additionally, the trend towards establishing bioeconomies around the world is demanding smarter treatment solutions. One such agricultural side stream produced in large quantities is oat hulls, i.e. the shell protecting the edible oat grain. In 2018, 23 million tons of oat were produced worldwide [2]. As the hull makes up 25–35% of the entire grain [3], considerable amounts of oat hull waste have to be treated.

Oat hulls like many other agricultural side streams are mainly composed of lignocellulose [4]. Lignocellulose is a complex, inter-connected network of cellulose, hemicellulose and lignin. Cellulose is a linear polymer composed of β -1,4-linked D-glucose units arranged in microfibrils. Hemicellulose is heterogeneous and includes branched polymers arranged in random and amorphous structures. In oat hulls, hemicellulose is mainly xylan, which is composed of a xylose backbone with limited substituents of arabinose, galactose, uronic and phenolic acids [4]. Lignin is an amorphous organic polymer composed of phenylpropane units differing in methoxyl group substitutions on the aromatic rings [1, 5]. The exact composition of lignocellulose varies greatly depending on the biomass as well as growth conditions [4]. Oat hulls consist of approximately 23% cellulose, 35% hemicellulose and 25% lignin under regular growth conditions. The hemicellulose is mostly arabinoxylan with few side chain substitutions [4]. The three main components in lignocellulose are strongly interconnected via various types of linkages. Cellulose is mostly connected to hemicellulose via hydrogen bonds, while lignin is covalently attached to hemicellulose via phenolic acid ester-ether bridges [5]. This complex structural organization alongside with its heterogeneous chemical content makes lignocellulose very resistant to chemical and biological decomposition, i.e. recalcitrant [1, 6]. Hence, efficient processing methods that are tailor-made for the respective biomass are required to overcome its recalcitrance and enable further use. These processing methods are typically referred to as pre-treatments.

Traditionally, pre-treatments were mostly explored with the aim of increasing the accessibility of cellulose for the production of biofuels [1, 5, 7]. Hence, they were evaluated on their efficacy to remove and break down hemicellulose and lignin. The societal pressure to move towards a stronger bioeconomy manufacturing various products from biomass as well as recent food trends demanding

fibre-enriched food products require the valorization of not only the cellulose, but also the hemicellulose and lignin fractions. This requires a reassessment of the previously evaluated pre-treatment techniques. Oat hulls are exceptionally rich in hemicellulose (35% of total dry weight), which makes them an excellent starting material for the production of dietary fibres. In order to exert beneficial prebiotic effects in the gut, they need to be solubilized [8]. Therefore, the suitability of a variety of pre-treatment techniques for the solubilisation of hemicellulose was evaluated in this study.

The different pre-treatment types are often classified according to their mode of action. The main groups are physical, chemical, physico-chemical and biological techniques [1, 5, 7]. The physical pre-treatments assessed in this study are milling, microwaving and ultrasonication. Milling is commonly used in combination with other pre-treatments as it increases the surface area by reducing particle size, which allows greater accessibility for further modification [1]. The irradiation of lignocellulosic biomass with microwaves weakens its recalcitrance by generating heat as well as vibrations of polar molecules leading to substantial collisions in a uniform way throughout the entire sample [7]. Ultrasonication causes a cavitation destabilization of the lignocellulose matrix by both generating oxidizing radicals (sono-chemical effect) and altering the surface structure (mechano-acoustic effect) [7]. As chemical pre-treatment methods, the effect of alkali and deep eutectic solvents (DES) was investigated. When biomass is incubated in alkali reagents the cellulose tends to swell, breaking its crystalline structure, which generates a greater porosity and exposes a larger surface area. Additionally, ester linkages, and to a lower degree ether linkages, between hemicellulose and lignin are broken under alkaline conditions, which significantly increases their solubility and leads to a decrease in the degree of polymerization [5, 7]. In contrast, DES have also been shown to efficiently solubilize hemicellulose (xylan) by disrupting intermolecular hydrogen bonds between the polysaccharides, which leads to an overall structural destabilization [9]. They are eutectic mixtures of a hydrogen bond donor and a hydrogen bond acceptor with strong hydrogen bond interactions. Due to their structural resemblance to the more established ionic liquids (ILs), they are often classified as a new group of ILs. The physico-chemical pre-treatment techniques are often based on stability differences of the individual lignocellulose components when exposed to thermal or pressure stresses. In this study, autoclaving was used to induce hydrothermal stress. Hemicelluloses are known to have a lower thermal stability than lignin and cellulose. They can therefore be solubilized at certain elevated temperatures, while cellulose and lignin remain intact [5]. Biological pre-treatments are still in their infancy, however positive results have been achieved with a laccase, which

oxidizes the lignin and hence makes the other components more readily accessible [1]. No biological pre-treatments were assessed in this study as the focus was placed on non-degrading techniques aiming at only solubilizing the material. For the same reason, no acid pre-treatment was tested as chemical pre-treatment.

The objective of the present study was to find a suitable pre-treatment method and optimize its conditions for maximizing the solubilisation of hemicellulose in oat hulls. This processing step is crucial for the production of hemicellulose fibres with prebiotic potential. Implementing such an application in industry would support the shift towards establishing a bioeconomy.

Materials and Methods

Raw Materials and Chemicals

Two different oat hull batches supplied by Lantmännen ek. för. were used in this study. Both batches were a mixture of Kerstin and Galant varieties grown in central Sweden (Mälaren Valley, with minorities coming from Östergötland (English exonym East Gothland) and Västergötland (English exonym West Gothland)), but in different years: 2017 (Swe17) and 2019 (Swe19). All seeds were obtained from SW-Seed (Sweden). Due to the different environmental conditions during the growth phase, the hemicellulose content in the batches differed, being 35.1% in Swe17 and 29.6% in Swe19 [4, 10]. In both batches, the hulls were industrially separated from the grains utilizing a Bühler BSSA stratopact HKE50HP-Ex peeler (Höflinger Millingsystems) and milled with an industrial size hammer mill (milling capacity of 1 t/h) located at the Lantmännen facilities in Järna.

For starch removal, the enzyme α -amylase from *Bacillus licheniformis* (type XII-A) was obtained from Sigma-Aldrich. All chemicals were purchased from Merck (Sweden) unless otherwise specified.

Autoclave Pre-treatment

The suitability of using an autoclave pre-treatment for the solubilisation of oat hull hemicellulose was assessed on destarched and milled Swe17 oat hulls. Destarching was performed according to the protocol by Sajib and colleagues [11]. Subsequently, the hulls were dispersed in Milli-Q water at a ratio of 1:10 (w/v) and autoclaved at 121 °C for 15 h (Inspecta, Thermo Scientific). After treatment, the liquid phase containing the solubilized material was separated from the solid phase via centrifugation at 3893×g for 20 min. The experiment was performed in six replicates.

Microwave Pre-treatment

For testing the microwave pre-treatment, 20 g of destarched and milled Swe17 oat hulls were dispersed in 160 mL Milli-Q water in a microwave beaker. The beaker was tightly closed and fixed in the sample holder of an Ethos PLUS 2 microwave (Milestone). The microwave was set to heat to 180 °C in 10 min and hold the treatment temperature for 30 min. After microwaving, the liquid phase was collected by centrifugation at 3800×g for 30 min. The treatment was run in duplicate.

Ultrasonication Pre-treatment

Ultrasonication was tested for its suitability of solubilizing oat hull hemicellulose. Destarched and milled Swe19 oat hulls were dispersed in Milli-Q water at a ratio of 1:5 (w/v) and placed in a Labassco Sonorex RK100H ultrasonic bath (Bandelin) with a frequency of 35 kHz for 30 min. Subsequently, the liquid fraction was separated via centrifugation at 3893×g for 10 min. The experiment was performed in duplicate.

Deep Eutectic Solvents Pre-treatment

Two different deep eutectic solvents (DES) were tested for their capability in solubilizing hemicellulose in oat hulls: choline chloride:glycerol ([Chol]Cl:Gly) and choline chloride:ethylene glycol ([Chol]Cl:Ethyl) (molar ratio 1:2). The solvents were prepared by continuously stirring the reagents at 80 °C in an oil bath until a homogenous clear liquid was formed. Destarched and milled Swe17 oat hulls were mixed with either of the DES at a ratio of 1:16 (w/v) and incubated at 115 °C for 3 h under constant shaking. After treatment, the liquid and solid fractions were separated via centrifugation at 3893×g for 20 min. Both treatments were performed in triplicate.

Alkali Pre-treatment

The effect of alkali incubation on the solubilisation of hemicellulose in oat hulls was tested with both sodium hydroxide (NaOH) and potassium hydroxide (KOH). Destarched and milled Swe17 oat hulls were dispersed in 2 M of either base at a ratio of 1:5 (w/v) and incubated at 40 °C for 16 h with continuous shaking. Subsequently, the liquid phase was separated from the solid phase via centrifugation at 3893×g for 20 min. The supernatant was neutralized with 37% hydrogen chloride to a final pH of 5–6. Both treatments were run in duplicate.

Combined Ultrasonication and Alkali Pre-treatment

The effect of combining ultrasonication and alkali pre-treatments was tested on destarched and milled Swe19 oat hulls. Five different types of treatments were performed in duplicate with a total treatment time of 2 h. In each treatment the oat hull to liquid ratio was 1:5 (w/v). In two treatments, the oat hulls were incubated at 40 °C for 2 h under constant shaking in either Milli-Q water or 2 M NaOH (control 1 and 2). In another two treatments, the oat hulls were first sonicated in a Labassco Sonorex RK100H ultrasonic bath (Bandelin) with a frequency of 35 kHz for 30 min and then incubated at 40 °C for 1.5 h while being immersed in either Milli-Q water or 2 M NaOH (control 3 and 4). In the final treatment, the oat hulls were first sonicated for 30 min in Milli-Q water, then the water was separated by centrifugation at 3893×g for 10 min and discarded. The remaining pellet was immersed in 2 M NaOH and incubated at 40 °C for 1.5 h. The final liquid fraction after all treatments was isolated using centrifugation at 3893×g for 10 min.

Design of Experiment Studies

In order to determine the best conditions for extraction and solubilisation of hemicellulose from oat hulls, two design of experiment studies (DOEs) were carried out on milled Swe19 oat hulls. The studies were planned and statistically analysed with the software MODDE 12.1 (Sartorius Stedim Data Analytics AB) based on a reduced central composite face design (DOE 1) and a central composite orthogonal design (DOE 2). The individual experiments were carried out following the procedure for the final treatment type described in section ‘[Combined Ultrasonication and Alkali Pre-treatment](#)’, but the factors ultrasonication length, temperature, NaOH concentration and NaOH extraction time were varied. A summary of the factor settings used in both studies is given in Table 1.

Soluble Hemicellulose Analysis

The solubilized fibres in the final liquid fractions of all pre-treatment types were precipitated by addition of four volumes of 99.5% ethanol and incubation at 4 °C overnight. After precipitation, the ethanol was removed by centrifugation at 3893×g for 5 min and evaporation. The dry fibres were weighed for yield determination.

The extracted fibres of the DOE studies as well as the optimized treatment were further characterized for their hemicellulose content following the NREL Laboratory Analytical Procedure (NREL/TP-510-42618, 2012). Extracted monosaccharides were identified and quantified utilizing HPAEC-PAD (ICS-5000, Dionex, Thermo Scientific) equipped with a CarboPac PA20 analytical column

Table 1 Factor settings for the design of experiment studies showing the minimum and maximum values for each factor, which enclose the design space. In DOE 2, the factors temperature and NaOH extraction time were kept constant at 80 °C and 9 h, respectively

Factor	Unit	Minimum	Maximum
<i>DOE 1</i>			
Ultrasonication length	min	10	60
Temperature	°C	20	80
NaOH concentration	M	0.5	5
NaOH extraction time	h	0.5	15
<i>DOE 2</i>			
Ultrasonication length	min	1	10
NaOH concentration	M	4	10

(150 mm×3 mm, 6 µm) as well as a respective guard column (30 mm×3 mm) as previously described by Falck et al. [12]. The starch content of the extracted fibre samples was determined using the “Total Starch” kit from Megazyme. Protocol K TSTA 09/14 method a was followed.

The phenolic acid content of both the untreated Swe19 batch as well as the extracted fibres from the same batch were analysed according to the method described by Sajib et al. [11].

Results and Discussion

Screening of Pre-treatment Methods

For the extraction and solubilisation of hemicellulose from the oat hull lignocellulosic matrix, a variety of common physical, chemical and physico-chemical pre-treatment methods were tested. Milling was performed on all oat hull material prior to processing with the other pre-treatment types. To assess the potential and allow comparability of the very different methods, rather harsh conditions were chosen for all of them. Several reaction conditions for the microwave and deep eutectic solvents treatments were tested, however, only the best results are presented here. The amounts of extracted and solubilized material based on oat hull dry weight are shown in Fig. 1. It has to be noted that this extracted material was not further characterized regarding its chemical composition, meaning that it most likely contains a mixture of cellulose, hemicellulose and lignin. Under the investigated conditions, both types of alkali treatments by far solubilized the most oat hull material (29% by KOH and 24% by NaOH). All other pre-treatments resulted in yields lower than 5%. This demonstrates the great recalcitrance of oat hulls as much higher results could be achieved with other lignocellulosic materials using the same methods (see Table 2).

Fig. 1 Extraction results of various pre-treatments showing the amount of solubilized material as a fraction of the destarched and milled oat hulls in percent (dry weight solubilized material of total

dry weight). The replicate numbers are reported as n. The two treatments, which were only performed once, are marked with an asterisk (*)

Table 2 Comparison of extraction results of the tested pre-treatment methods showing the amount of solubilized material (or fractions if further specified) from the destarched and milled oat hulls in this study and similar lignocellulosic materials in other studies. All numbers are given as percent

Pre-treatment method	Yield of solubilized material from oat hulls [%]	Yield of solubilized material from other lignocellulosic materials [%]	References
Autoclave	3.5	5.7 (brewer's spent grain) 17.2 of arabinoxylan fraction	[11]
		54 (rye bran) 45 of arabinoxylan fraction	[12]
Microwave	4.4	50.8 (wheat bran) 48 of arabinoxylan fraction	[13]
		57 (brewer's spent grain) of hemicellulose	[14]
Ultrasonication	0.1	14.7 (corn bran) of polysaccharides	[15]
		5.1 (sugarcane bagasse)	[16]
Deep eutectic solvents	1.0 ([Chol]Cl:Gly) 0.8 ([Chol]Cl:Ethyl)	92 (E. globulus wood) of xylan	[9]
Alkali	23.8 (NaOH) 29.0 (KOH)	32.3 (sugarcane bagasse)	[17]
		67.2 (rice straw) of hemicellulose 88.5 with H ₂ O ₂	[18]

Autoclaving was not a successful approach to solubilise hemicellulose from the lignocellulose matrix, and was limited to conditions that did not degrade the hemicellulose which is the least thermostable polymer in the lignocellulose complex. Targeting of the hydrogen bond interactions by DES, did also not result in solubilisation. Thus, it seems that the breakage of covalent bonds binding hemicellulose into the oat hull matrix is crucial for hemicellulose solubilisation. To achieve this, three methods were included in the screening: ultrasonication, microwave treatment and alkaline treatment. Neither ultrasonication, nor microwave treatment led to significant solubilisation. The oxidizing radicals generated

during ultrasonication and the collisions of polar molecules caused by microwave irradiation seem to not be sufficient enough to break these covalent bonds. Alkaline treatment was more successful, and has the advantage of both breaking ester and ether bonds connecting the hemicellulose to lignin as well as increasing porosity by cellulose swelling, which allows the reagents to penetrate the entire material. However, the alkaline conditions need to be rather harsh as a previously established alkaline hydrogen peroxide treatment method, utilized for other types of biomass materials [5], did not solubilize much material or alter the oat hull's lignocellulosic composition [10].

Combined Pre-treatments Using Ultrasonication and Alkali

The effectiveness of alkali pre-treatment has been reported to be significantly increased when combined with other types of pre-treatment [5]. One successful combination is the sequential application of ultrasonication followed by alkali treatment. On sugarcane bagasse, this combined treatment increased the yield as ultrasonication led to a mechanical disruption of the cell wall resulting in an increased accessibility of the material to alkali attack [16]. Therefore, the effect of combining ultrasonication with alkali pre-treatment for the solubilisation of oat hull hemicellulose was tested. Even though slightly higher values were obtained for the KOH treatment in the screening trial (Fig. 1), all further tests were performed with NaOH due to its easy availability for industrial scale treatments.

The results of this combined extraction are visualized in Fig. 2. Alongside the preferred sequential ultrasonication in water followed by incubation in NaOH, several alternatives (controls) were included in the study, involving varying the solvent during the ultrasonication or incubation step. It can be seen that incubation in water only (during 2 h) (control 1) as well as ultrasonication in water with subsequent incubation in water during a corresponding period of time (control 3) were not capable of solubilizing the oat hull hemicellulose. Incubation of the oat hulls in NaOH (control 2), on the other hand, did dissolve a notable fraction, despite use of a shorter incubation period (2 h) than in the screening (16 h, Fig. 1). However, this yield could not be increased substantially when the oat hulls were first sonicated in NaOH, followed by incubation in the same solvent (control 4). This stands in contrast to previous studies on wheat straw and corn bran, which showed positive effects

on the hemicellulose extraction yield when sonicated in an alkali medium [15, 17]. The present study however demonstrates that the yield of extracted material was nearly tripled (combined extraction in Fig. 2) when the material was first ultrasonicated in water, followed by replacement of the water by alkali and incubation at the same temperature and time as used in control 4.

The cavitation events triggered by ultrasonication that lead to the degradation of biomass are strongly influenced by the solvent the material is dispersed in. One important solvent parameter influencing the number of triggered cavitation events is viscosity [19]. NaOH solutions have a higher viscosity than water, becoming more pronounced with increasing NaOH concentration. Ultrasonication in control 4 in the present study was performed in a more concentrated NaOH solution than in the study showing good hemicellulose extraction from corn bran [15]. Therefore, fewer cavitation events might have been generated in this study producing fewer radicals and less extreme high local temperature and pressure effects. It furthermore hints at a more recalcitrant lignocellulose structure in oat hulls compared to corn bran. Ultrasonication in water alone has been shown to mainly solubilize heavily substituted xylan from sugarcane bagasse due to the lower amount of possible interactions with cellulose [16]. As oat hull hemicellulose contains very few side chain substitutions, its solubilisation must require harsher conditions explaining why ultrasonication alone failed to solubilize much material. However, the results in Fig. 2 show that it must destabilize the material in a way that enables easier solubilisation by a subsequent alkali treatment.

Fig. 2 Extraction results of the combined ultrasonication and alkali pre-treatment trial as well as four controls showing the amount of solubilized material as a fraction of the destarched and milled oat hulls

in percent ($n=2$). A short description of the treatment of all samples is given to the right of the graph

Pre-treatment Method Optimization

First Design of Experiment Study

Based on the promising results of the combined ultrasonication and alkali extraction screening study, a design of experiment study (DOE) was carried out to determine the optimal conditions for hemicellulose solubilisation. Contrary to the analysis in the screening studies, the extracted material was analysed regarding its lignocellulose composition and the yield numbers reported here only include the extracted hemicellulose fraction with respect to the whole oat hull sample. Additionally, the destarching step prior to extraction (used in the screening trials above) was omitted as the starch content in the sample was experimentally found to be low enough (12%) to not influence the extraction. Based on the screening trials, the factors selected to evaluate the influence on the extraction yield were ultrasonication length, NaOH concentration, NaOH incubation time and NaOH incubation temperature. A reduced central composite face design was determined to be the most suitable for analysis as it allows the analysis of second order terms (quadratic) for four factors while minimizing the necessary number of experiments. Following the suggestion by the modelling software MODDE, 23 extractions varying the factor values were carried out. The yield results from those extractions generated a model with high summary of fit parameters ($R^2=0.87$; $Q^2=0.76$; Model validity = 0.78; Reproducibility = 0.89), which indicate that the created model fits well to the experimental data. With the factor settings applied in the DOE 1 design space (Table 1), the model suggests that the ultrasonication length does not have an effect on hemicellulose solubilisation, indicating that the minimum of 10 min of ultrasonication might be sufficient for the effect seen in the screening test (see Fig. 3A). Both NaOH concentration and incubation temperature are very important, however the optima are lying outside the design space at the higher ends, suggesting that even higher concentrations and higher temperatures might yield better results. NaOH incubation time is also a very important factor and the optimum was found within the design space at 8.96 h. The optimizer function of the software suggested a maximum yield of 21.9% solubilized hemicellulose relative to the entire oat hull under the following conditions: 55 min of ultrasonication, 5 M NaOH concentration, 80 °C incubation temperature and 9 h incubation time. To verify the validity of the model, a triplicate experiment at exactly these conditions was carried out. The result was a yield of $21.3 \pm 1.3\%$, which is very close to the predicted value. Therefore, the model seems to be corresponding to reality very well.

As not only hemicellulose is solubilized during the treatment, but also lignin and cellulose, the DOE factors were also assessed for their influence on hemicellulose purity in

the extracted material. As shown in Fig. 3B, the purity in general is rather low yielding a maximum of 0.22, i.e. 22%, at the highest NaOH concentration, lowest incubation temperature and shortest incubation time. At those conditions, the hemicellulose yield (4.8%) is very low. This suggests the presence of a small fraction of easily accessible, potentially more substituted, hemicellulose that can be solubilized at milder conditions. The major fraction, however, seems to be very strongly anchored to the lignocellulosic matrix requiring some degradation and solubilisation of lignin and cellulose for its liberation. As the main aim of the study is to maximize the amount of soluble hemicellulose, the following optimizations only regarded hemicellulose yield and not its purity.

Second Design of Experiment Study

In order to increase the design space and evaluate extractability of oat hull hemicellulose at shorter ultrasonication times and higher NaOH concentrations, a second DOE study was designed. Based on the results of the first DOE, the treatment time was fixed at the optimal 9 h of incubation and the incubation temperature at 80 °C as higher temperatures require specialized equipment and might not be suitable for large scale production. Ultrasonication times of 1 to 10 min and NaOH concentrations of 4–10 M were investigated in a central composite orthogonal design allowing the analysis of second order terms (quadratic) for the two factors in 11 experiments. The generated model fits well to the experimental data as shown by the high summary of fit parameters: $R^2=0.96$; $Q^2=0.86$; Model validity = 0.75; Reproducibility = 0.97. In this design space (DOE 2, Table 1), the extraction yield was very strongly dependent on ultrasonication time, which also had a significant interaction term with NaOH concentration. The model suggests that the ultrasonication time should be kept low at lower NaOH concentrations and high at higher NaOH concentrations (see Fig. 4). However, all results, even the optimum (19.1%), in this design space are below the optimum in the previous study. Therefore, it can be concluded that shorter ultrasonication times and higher NaOH concentrations do not increase the yield. The higher NaOH concentrations might also lead to a degradation of the polysaccharides instead of further solubilisation.

Final Method Adjustments and Analyses

The DOE studies suggest that the optimal conditions for hemicellulose solubilisation are incubation at 80 °C with 5 M NaOH for 9 h. However, it remains unclear, if a shorter ultrasonication time of 10 min yields as much as the predicted best setting at 55 min. Therefore, a final study was performed in which only the ultrasonication length was

Fig. 3 Response contour plot showing the influence of ultrasonication time, NaOH concentration, incubation time and incubation temperature on the hemicellulose solubilisation yield (**A**) and hemicellulose purity in the extracted material (**B**) in the first DOE study

varied followed by the same optimal conditions of all other parameters. The results displayed in Fig. 5 show that there are no large differences between the yields of solubilized hemicellulose among all the sonicated samples. This shows that a 10 min long ultrasonication step is sufficient.

For further chemical characterization of the soluble material obtained with this final optimized extraction method, a larger batch starting with 25 g of the milled oat hulls was produced. The resulting soluble material is a heterogeneous mixture of many components (see Table 3). Of the initial

oat hull starting material 24.6% of the lignocellulose components were solubilized. The largest fraction was hemicellulose (22.1% of total dry weight) followed by starch (11.9%) and lignin (2.5%). This corresponds to a solubilisation of 74.7% of all available hemicellulose in the oat hull. This yield is significantly higher than results from previous similar extraction protocols combining ultrasonication and alkali treatments on wheat straw (65% yield of hemicellulose) [17] and corn bran (32% yield of hemicellulose) [15] for hemicellulose solubilisation, exemplifying

Fig. 4 Response contour plot showing the influence of ultrasonication time and NaOH concentration on the hemicellulose solubilisation yield in the second DOE study

Fig. 5 Extraction results of ultrasonication length trial showing the amount of solubilised hemicellulose as a fraction of the oat hull sample. All tests were performed in duplicate

the importance of optimizing reaction parameters as well as choosing the best pre-treatment method for the raw material under investigation.

Additionally, the phenolic acid content before and after extraction was analysed. Of special interest is ferulic acid as it acts as a linker between the individual hemicellulose strands as well as between hemicellulose and lignin, significantly contributing to the recalcitrance and insolubility of the material [5]. The extracted material contains 20 times less ferulic acid than the untreated oat hulls (see Table 4), showing that the developed pre-treatment method is very

efficient in removing these structures. The greater solubility of the pre-treated material is therefore (at least partly) due to the breakage of intermolecular connections between individual hemicellulose molecules and hemicellulose and lignin molecules. Similarly, a 15 times reduction of all phenolic acids was observed after the pre-treatment suggesting that the developed method is effective in breaking ester bonds.

For successful application of this extraction and solubilisation process in industry, the method needs to be up-scalable. The greatest challenge here is to find appropriate equipment. The alkali treatment stage can easily be implemented

Table 3 Quantities of the major components in the untreated Swe19 oat hulls, in the solubilized material after pre-treatment (combined ultrasonication and alkali extraction) in percentage of the original material as well as the solubilized amount related to the original amount present in the untreated oat hulls

Component	Untreated [%]	Solubilized material [%]	Solubilized fraction of original material [%]
Cellulose	15.7 ± 3.0	0	0
Hemicellulose	29.6 ± 2.0	22.1 ± 3.4	74.7
<i>Arabinose</i>	4.2 ± 0.2	3.4 ± 0.6	81.0
<i>Galactose</i>	1.6 ± 0.2	1.0 ± 0.2	62.5
<i>Xylose</i>	23.3 ± 1.2	17.5 ± 2.7	75.1
<i>Mannose</i>	0.5 ± 0.4	0.2 ± 0.0	40.0
Lignin	21.8 ± 0.1	2.5 ± 1.8	11.5
Starch	12.1 ± 0.5	11.9 ± 0.5	98.3

The two DOE labels in the graph were displayed as italics as means of visual separation from the columns. They represent two breaks in the table that do not follow the column structure

Table 4 Phenolic acid content in Swe19 oat hull batch before and after combined pre-treatment of ultrasonication and alkali extraction. The total phenolics content is reported as area underneath all peaks in the HPLC chromatograms due to lacking standards for all phenolic acids

Swe19 batch	Ferulic acid content [µg/g]	Total phenolics [mAU*min]
Before Pre-treatment	2840 ± 447	152 ± 20
After Pre-treatment	141 ± 137	10 ± 7

as large scale alkali processing is commonly employed in pulp mills. For this reason it has previously been judged to be the most competitive pre-treatment technique [20]. Large scale ultrasonication applications are not as widespread, however, many efforts to establish it have been made in recent years [19] providing a promising outlook for the application of ultrasonication-assisted alkaline extraction on large scale.

Conclusion

Ultrasonication-assisted alkaline extraction for the solubilisation of hemicellulose in oat hulls was shown to be a very suitable pre-treatment method. The optimal treatment conditions are 10 min of ultrasonication in water, followed by a 9 h long incubation in 5 M NaOH at 80 °C. At these conditions, 74.7% of the hemicellulose present in the raw material was solubilized.

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Declaration

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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