ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Hot-Water Hemicellulose Extraction from Fruit Processing Residues

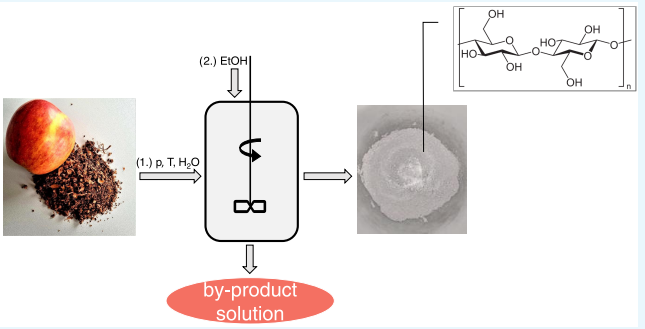
Marius Wolf, Frederik Berger, Stefan Hanstein,* Anke Weidenkaff, Hans-Ulrich Endreß, Arne Michael Oestreich, Mehrdad Ebrahimi, and Peter Czermak

Cite This: *ACS Omega* 2022, 7, 13436−13447

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Hemicelluloses are an abundant biopolymer resource with interesting properties for applications in coatings and composite materials. The objective of this investigation was to identify variables of industrially relevant extraction processes that increase the purity of hemicelluloses extracted from fruit residues. Our main finding is that extraction with subcritical water, followed by precipitation with alcohol, can be adjusted to yield products with a purity of at least 90%. Purity was determined based on the total concentration of glucose, galactose, xylose, arabinose, and mannose after hydrolysis with sulfuric acid. In the first experimental design (DoE methodology), the effects of extraction temperature (95−155 °C) and time (20−100 min) on yield and purity were studied. A clear trade-off between yield and purity was observed at high temperatures, indicating the selective removal of impurities. In the second experimental design, the influence of extract pH and alcohol concentration on yield and purity was investigated for the raw extract and a concentrate of this extract with 1/6 of the original volume. The concentrate was obtained by ultrafiltration through ceramic hollow-fiber membranes. The highest purity of 96% was achieved with the concentrate after precipitating with 70% alcohol. Key factors for the resource efficiency of the overall process are addressed. It is concluded that extraction with subcritical water and ultrafiltration are promising technologies for producing hemicelluloses from fruit residues for material applications.



INTRODUCTION

Polymeric sugar products are established bulk materials in the chemical industry. For historical reasons, starch and chemical pulp are readily available raw materials for a diverse range of chemical modifications with multiple applications in the paper, construction, cosmetics, food, and pharmaceutical industries and others. In the field of functional polymers, starch and its chemical derivatives represent a worldwide market volume of 12 million tons.[1] A large molecular weight is favorable for several applications (e.g., for imparting high viscosity at low concentrations), but in resin applications, lower molecular weight performs better. In coating resins and printing inks, petrol-based binders with a molecular mass between 1 and 10 kDa are required.[2] Furthermore, in many cases, sugar polymers with large molecular weight require intensified treatments (e.g., mechanical force) to bring about the required degree of chemical substitution. Thus, for an application in which large molecule size is not required, lower conversion costs would favor the use of these polymers. Plant biomass contains sugar structures, which are closer to the specified size requirement—hemicelluloses—which are increasingly being recognized as a future source for material and coating applications.[3−6] Hemicelluloses represent an abundant and diverse group of plant-derived ß-glycosidic sugar structures, which easily fulfill the basic requirements for binders in coating systems: dispersible in aqueous solvents, film-forming, and carrying multiple reactive groups as a prerequisite for intensive cross-linking.

Methods for industrial-scale extractions of hemicelluloses can be divided into extractions with alkali solutions and with pressurized hot water (subcritical water). For recalcitrant woody biomass, alkali extraction has been proven to provide hemicelluloses with good yield and purity.[7] Alkali extraction of hemicelluloses as pretreatment of wood chips is compatible with kraft pulp production, as long as sufficient hemicelluloses remain in the pulp, since hemicelluloses contribute to the mechanical strength of paper.[8] With alkali extraction at temperatures below 100 °C, hemicelluloses with a molar mass in the order of 20.000 g/mol are frequently reported.[7] Since acetyl groups are saponified at alkaline pH, polymer chains become more uniform, improving their adhesion to

Received: October 29, 2021
Accepted: March 18, 2022
Published: April 12, 2022

pulp fibers, which improves mechanical pulp properties.[9] Alkaline hemicellulose extract solutions contain a considerable amount of lignin. To purify hemicelluloses, the pH is lowered to a defined value in the range between 7 and 4, and hemicelluloses are precipitated with alcohol, while lignin remains soluble.[10] Precipitation with acid is less efficient. For alkaline extracts from lignocellulosic tissues from four hardwoods and switchgrass, the hemicellulose yields were lower when acid was used for precipitation instead of alcohol.[11] To extract a major fraction of the hemicelluloses from woody biomass, in which hemicelluloses and lignin are tightly interconnected, delignification is required as a pretreatment, rendering the alkaline extraction method less eco-friendly. Moreover, during neutralization of the alkaline crude extract, inorganic salts accumulate, which causes additional process costs for separating them from the product, e.g., by dialysis, and from the residual liquid. This is not only relevant for biorefinery concepts for kraft pulp mills but also for organosolv biorefineries.[12] Delignification is not necessary when alkali is applied at a temperature of 121 °C to fine eucalyptus wood powder (sawdust), but thorough washing of the hemicellulose product with water is essential to remove lignin, which has been coextracted with alkali.

Extraction with water has the advantage that chemicals are not required and salt byproducts do not occur. It has been an exceptional business case that hemicelluloses (arabinogalactans) from larch wood could be extracted with warm water at a high yield (30% of wood biomass), which allowed cost-competitive commercial production during the era of rapid growth of the fossil-based polymer industry.[13] Most raw materials are more recalcitrant regarding hemicellulose extraction. When water is heated to above 100 °C in a closed vessel, the pressure increases, the physicochemical water properties change (increase of the ionization constant, decrease of surface tension, dielectric constant, viscosity), and water becomes a good solvent for hemicelluloses. At the same time, hydrolysis of glycosidic bonds is favored. However, with appropriate process design (see below), xylans with a molar mass of ca. 20.000 g/mol and higher are obtained, e.g., from birch wood sawdust.[14] The molecular weight decreases by 90% during extraction for 120 min. With pressurized hot water, extraction conditions (pH, time, temperature, particle size, stirring/percolation) have to be carefully controlled to prevent an overshoot of hemicellulose autohydrolysis. The extraction and hydrolysis kinetics may largely differ between raw materials.[15,16] Furthermore, hydrolysis rate depends on coextracted compounds; e.g., solubilized lignin counteracts autohydrolysis.[17] Hot-water extraction of hemicelluloses was recently suggested as a process module of organosolv biorefineries, which can produce hemicelluloses and lignin in a more native state compared to lignin from the traditional pulp operations.[18]

This paper focuses on fruit residues of the food industry as a source of hemicelluloses. Biomass with a large proportion of nonlignified tissue, e.g., fruit pomace, contains more protein compared to wood. Many proteins are readily dissolved with alkali and precipitated with alcohol. With such raw materials, hemicelluloses prepared from alkali extracts may contain up to 30% protein (Hanstein, unpublished). Similar to phenolic impurities, proteins interfere with chemical modification reactions and with cross-linking of sugar building blocks in coating resins. Since for binder applications of hemicelluloses molecules with a molar mass below 10.000 g/mol are desired, which have to be produced at low cost with little protein contamination, extraction with pressurized hot water represents a flexible process platform for wood and non-wood biomass. However, producing hemicelluloses with a purity comparable to chemical pulp (≥90%) from a broad range of raw materials at a competitive price remains a huge challenge.

Hemicellulose extraction with subcritical water is governed by two main processes: solvation and hydrolysis/decomposition.[19] These processes also occur for the other components of the plant tissue which are coextracted. Extraction is combined with a selective precipitation process for the larger molecules in the raw extract. The purity of the precipitate will improve if hydrolysis/decomposition favors the conversion of dissolved impurities to products that are not precipitated. This paper reports on the effects of extraction temperature, extraction time, and alcohol concentration on the yield and purity of the precipitated hemicellulose products. Systematic investigations of these hemicellulose product properties for extraction with subcritical water from fruit residues are scarce. We used the design of experiment methodology, applying a central composite design. Our data provide novel evidence that intensification of the extraction improves the separation of hemicelluloses from coextracted solutes during the subsequent alcohol precipitation step. Furthermore, the influence of ultrafiltration (with concomitant strong volume reduction) before precipitation on the yield and purity of a hemicellulose product from hot-water extracts of fruit residues is demonstrated for the first time.

MATERIALS

Depectinized apple pomace was delivered as dry matter by Herbstreith & Fox GmbH & Co. KG (Neuenbürg, Germany). Bioethanol (100%) was purchased from Höfer Chemie GmbH. Sulfuric acid (96%), aqueous phenol solution (90%), D-glucose solution (100 g/l), D-glucose (≥99.5%), galactose (≥99%), mannose (≥99%), arabinose (≥99%) and xylose (≥99.0%) were bought from Sigma. Partially hydrolyzed tamarind seed gum was purchased from DSP Gokyo Food & Chemical Co., Ltd., Japan.

METHODS

Investigation of Extraction Parameters. The selection of parameter values for the investigation of the extraction process accounts for the plan to transfer results to a pilot-scale extraction plant from Schrader Verfahrenstechnik (Ennigerloh, Germany), which has been built up at the Fraunhofer facilities for hemicellulose extraction from food residues. It contains a stainless steel percolation extractor with a volume of 50 L, which is flushed with a flow rate of 25 L/min, a precipitation tank with a volume of 400 L, and a rectification column for recovery of the ethanol from the solution after hemicellulose precipitation. The closed extractor system can be operated at temperatures up to 150 °C and a pressure of up to 10 bar. The raw material is placed on the bottom of the extractor on a sieve plate with 1 mm holes. Due to the upper temperature limit of the extractor, the selected temperature range of this investigation was between 95 and 155 °C. Regarding the range of treatment times, the work of Anderez Fernandes[15] was instructive who has shown for three wood species that at 140 °C, average molar masses of hemicelluloses decrease to a level between 10 and 5 kg/mol, which is the relevant range for binder molecules in coatings (2). The extraction was

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

investigated with the parameter settings of a central composite design (DoE) with the values shown in Figure 1.

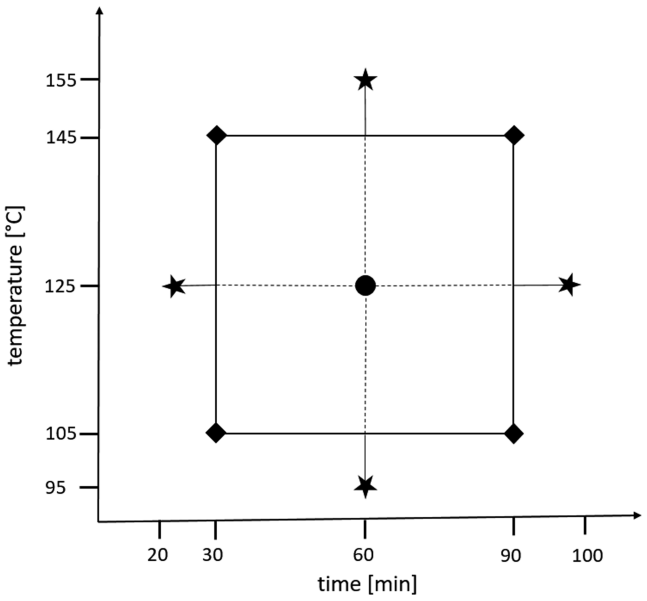


Figure 1. Statistical experiment design created using Design-Expert 13 to investigate the interactions between changing the temperature and extraction time to improve hemicellulose extraction in terms of yield and purity.

An autoclave (Büchi Polyclave, reactor volume 1.5 l, Büchi Glas Uster, Switzerland) was used. The biomass charge was 25 g of air-dried, depectinized apple pomace in a volume of 500 mL of distilled water. The particle size distribution of the apple pomace was determined by sieving 1 kg of material in triplicate with a sieve tower (mesh sizes from 800 μm to 12.5 mm). The resulting particle distribution is illustrated in Figure 2. Since the risk of blocking the sieve plate in the pilot-scale extractor increases and separation of solid extraction residues from the liquid phase becomes more difficult with smaller particles, particle size was left unaltered. The suspension was vigorously

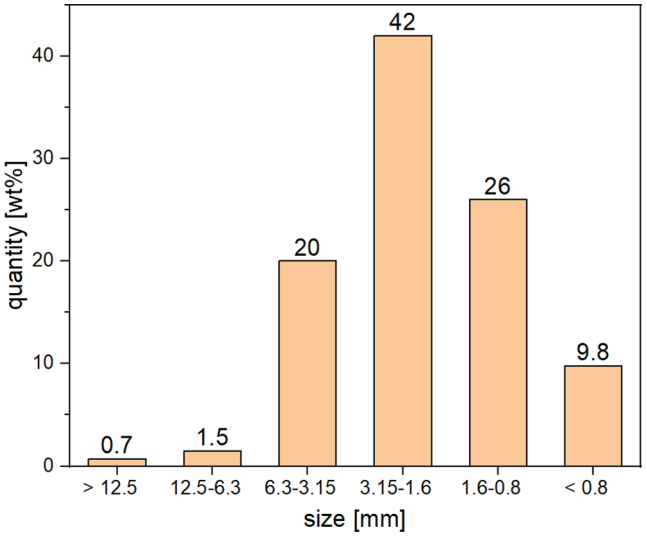


Figure 2. Statistical particle size distribution of air-dried apple pomace determined by sieve tower with mesh sizes from 800 μm to 12.5 mm.

stirred at 200 rpm. For the initial heating phase, a heating rate of 4 °C min^{-1} was chosen.

Before releasing the extract from the autoclave, it was cooled down to 40 °C. After extraction, the product was precipitated with pure ethanol at room temperature overnight with a resulting concentration of 70% (v/v) ethanol.

Investigation of Precipitation Parameters. Precipitation was investigated with the parameter settings of a central composite design (DoE) with the values shown in Figure 3.

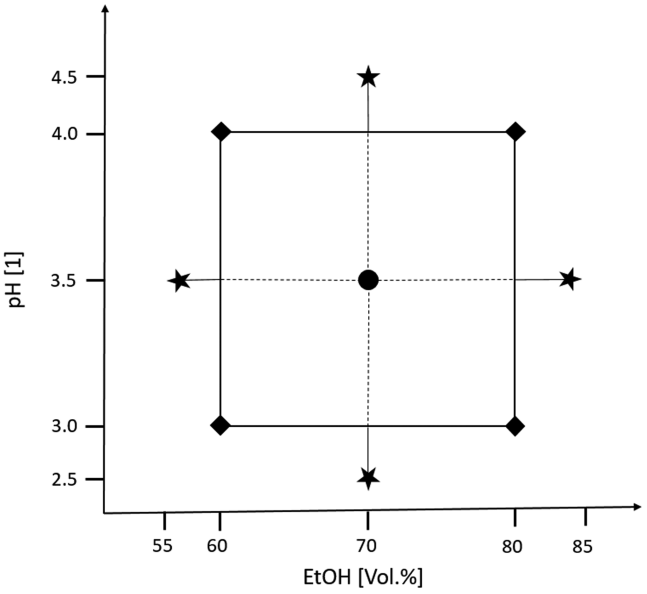


Figure 3. Statistical experiment design created using Design-Expert 13 to investigate the interactions between changing pH value and ethanol concentration to improve hemicellulose precipitation from glycan solution.

Aqueous ethanol solutions were added at a 4:1 volume ratio to yield the desired final ethanol concentration. For a final concentration of 70% (v/v), the ethanol concentration was 87.5%. This ratio was adapted from the pilot plant, where the ethanol concentration after the rectification process usually varies between 85 and 88% (v/v). The precipitated hemicelluloses were separated from the ethanol solution by centrifugation at 6185*g* (4700 rpm, Beckman Coulter Allegra X30-R centrifuge) for 5 min at 18 °C. In the experiments with different extraction conditions, two washing steps followed by centrifugation at 6185*g* (4700 rpm) for 5 min were performed. The first washing step was performed with 100 mL of an 80% (v/v) ethanol solution, the second with 100 mL of 100% ethanol. For the experiments at different precipitation conditions, the solid was washed with 80% (v/v) ethanol until the supernatant liquid was clear and colorless, followed by a final washing step with 100% (v/v) ethanol. The products were superimposed with 40% (v/v) ethanol before lyophilization (Christ Alpha 3-4 LSCbasic freeze-dryer with a VACUUBRAND Chemistry-Hybrid Pump RC6).

Acid Hydrolysis. For chromatographic sugar analysis, according to Willför et al.,[20] the sugar structures were hydrolyzed to the monomers in a two-step process according to Seaman et al.[21] Briefly, 20 mg of hemicellulose was prehydrolyzed for 30 min with 200 μL 72% sulfuric acid while being stirred and incubated at 30 °C. Then, 5.6 mL of ultrapure water was added (2.5% sulfuric acid), and the test

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

tube was placed in a microwave digester (turboWAVE, MLS-MWS Laboratory Solutions) for 60 min at 120 °C and 40 bar N_2. The hydrolysate was diluted to 50 mL with ultrapure water (0.28% sulfuric acid) and filtered through 0.45 μm syringe filters. For each extraction, hydrolysis was performed three times, and the hydrolysate was measured in duplicate. For the hydrolysis replicates, the relative standard error, i. e., the standard error relative to the mean value of the sugar concentration, was on average 5.41% for arabinose, 2.78% for galactose, 2.86% for glucose, 3.01% for xylose, and 5.06% for mannose (n = 8 treatments with subcritical water; the treatment at 95 °C for 60 min was excluded from the analysis of standard errors, since results of one hydrolysis were less than 50% compared to the other two replicates).

Ion Chromatography. The individual sugars were separated by high-performance-ion-chromatography with a Dionex CarboPac PA20 IC column (3 × 150 mm, Thermo Fisher) and quantified by pulsed amperometric detection (HPIC-PAD, ICS 5000$^+$, Thermo Fisher). Separation was isocratic with 2 mM NaOH. Ultrapure water (18.3 MΩ) was used for preparing eluents and standards for the individual sugars. 2-Desoxyglucose was added as an internal standard. Concentrations of internal standard was 25 mg/L. The calibration was conducted with five solutions with concentrations [mg/L]: 1.5625, 3.125, 6.25, 12.5, and 25 for each individual sugar.

Phenol Sulfuric Acid Method (PSA). The colorimetric assay with phenol as a coloring agent, as used by DuBois,[22] to quantitatively determine the presence of sugars, oligosaccharides, and polysaccharides, has already been used for single sugars as well as heterogeneous polysaccharide mixtures from plant biomass.[23−25] Therefore, 100 μL of aqueous test liquid with a carbohydrate concentration of 5 g/L was pipetted into a suitable vessel. Then, 100 μL of distilled water and 100 μL of 90% aqueous phenol solution were added. The vessel was placed in a 40 °C water bath and stirred properly with a magnetic stirrer. Then 4 mL of concentrated sulfuric acid was added rapidly, directly onto the liquid surface rather than against the walls of the vessel. The samples were incubated for 30 min in the water bath and cooled down to room temperature before measuring the UV−vis spectra (Cary 100, Agilent). The resulting chromophore generates orange-yellow color with maximum absorbance at 480 nm. The absorbance at 482 nm was used for calculating the sugar concentration as glucose equivalents. Blank solutions were prepared in the same manner, but the carbohydrate solution was replaced by distilled water.[22] Each sample was prepared in triplicate. The calibration curve was prepared with D-glucose dilutions between 0.5 and 5 g/L.

Ultrafiltration. To reduce the necessary ethanol volume for precipitation to 1/6, the volume of the glycan solution was reduced accordingly by ultrafiltration. THM Gießen performed the ultrafiltration using a setup containing a ceramic membrane (1 kDa) produced by Atech Innovations GmbH. The cutoff value was selected because the target was to retain oligomers with a molar mass down to 1 kDa. The technical characteristics of the membrane are given in Table 1.

The system was cooled with a circulation cooler at 10 °C, and the permeate and retentate were cooled with ice. Filtration was performed with 2.0 ms^{-1} CFV and 3.0 bar TMP within 11.5 h. Preliminary experiments have shown that with a lower TMP, foam formation decreases the flow and strongly increases membrane resistance.

Table 1. Technical Data of Ceramic Hollow-Fiber Membrane Used for Ultrafiltration

parameter	description
cutoff	1 kDa
number of hollow-fibers	37
hollow-fiber diameter	2.0 mm
cross-sectional area	0.000116 m^2
filtration area	0.098105 m^2
length of active layer	0.422 m
pH stability	0−14
maximum operating temperature	90 °C
maximum pressure	10 bar
material	Al_2O_3

Design-Expert 13 Processing. Design-Expert 13 was used as a mathematical tool to create the statistical design of the experiments and to analyze the data. The data were fitted with models suggested by the software. Suggestions were based on the p-value of each model, which indicates if the fit is significant, and on the p-value of the lack of fit, to indicate whether the deviation of the model from the measured data is significant. In this study, quadratic models were suggested, with the exception of yields in the precipitation experiments. Here, a linear model was suggested. Considering the correlation coefficients (adjusted R^2 and predicted R^2), another parameter was provided to evaluate the probability that the suggested model was appropriate. A difference of less than 0.2 between adjusted R^2 and predicted R^2 shows that the contour plot provides a good illustration of measured values.

The software output includes an ANOVA table where all factors and factor interactions are rated with p-values to show whether they have a significant effect on the results ($p < 0.05$). Further analysis was conducted within the diagnostics tab. These diagnostic options include a normal plot of residuals (we want the data to be as close to the line as possible to have it consistent), comparison of predicted vs actual plot (also for data consistency), the Box-Cox-plot to see if there would be any model transformation recommended, and the residuals vs predicted plot to make sure that the data are statistically distributed and do not follow a trend. Where optimal conditions were specified in the text, they were determined using numerical optimization based on the suggested model. Desired responses were maximized (yield and purity), while parameter values were changed, and the most suitable combination of parameter values was determined according to the selected preferences.

Energy Demand Analysis. The energy requirement of ultrafiltration for preparing a concentrated glycan solution with 1/6 of the original volume was compared to the energy demand for alcohol recovery from a 5-fold volume of aqueous alcohol solution, from which the alcohol has to be recovered when no ultrafiltration is performed. For the energy demand of ultrafiltration, the performance of the lab-scale pump was multiplied by filtration time. For alcohol recovery, the energy demand was derived from the rectification process in the IWKS extraction pilot plant (see the Methods section on Extraction), which is dominated by the energy consumption of the electrical heating unit.

RESULTS AND DISCUSSION

Parameters of Hot-Water Extraction. *Hemicellulose Extraction.* As illustrated in Figure 4, the extraction temper-

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

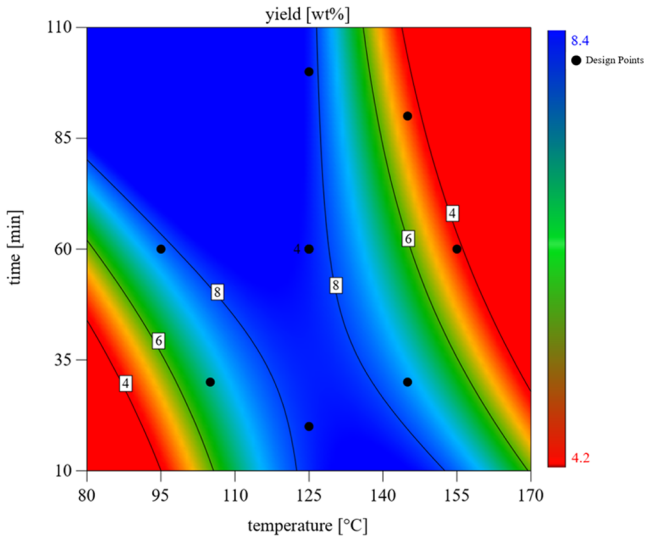


Figure 4. Overall yield related to the 25 g apple pomace: divided into low yield (red), medium yield (green), and high yield (blue).

ature and the interaction between temperature and extraction time had a significant influence on the overall yield. As the red area shows, yield remained low at extraction temperatures below 90 °C and above 140 °C. As indicated by the blue area, a percentage yield above 8% could be achieved with extraction temperatures between 100 and 140 °C (measured yield in Table A1). Statistical information on the influence of the factors is provided in Table A2. Up to a temperature of about 125 °C, higher yields were obtained by increasing the extraction time. At extraction temperatures above 125 °C, an increase in extraction time resulted in a loss of yield. The pH value before extraction was 4.5. After extraction, depending on the parameters, the pH value ranged from 3.92 (harsh conditions) to 4.2 (mild conditions).

Figure 5 shows the results of HPIC-PAD analysis for a hydrolyzed hemicelluloses sample after hot-water extraction. Since not all sugar units are converted to sugar monomers during sample preparation (glycan hydrolysis), commercially available purified tamarind seed gum was used as a reference. It

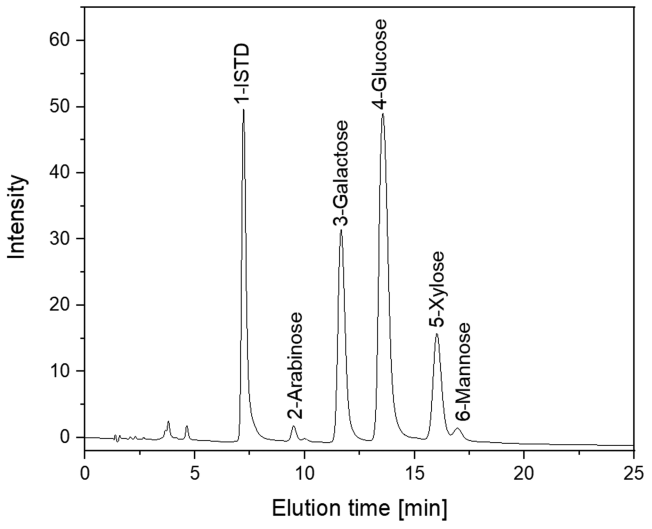


Figure 5. IC chromatogram measured of a hydrolyzed hemicellulose sample, showing the separation of monomeric sugars.

is a xyloglucan with a similar monomer composition (not shown) like the product from apple pomace.

The sugar concentration measured in tamarind seed gum was 77.4%. The concentration values from the apple pomace product are given in relation to the measured purity of tamarind seed gum (Figure 6). The lowest purity between 30

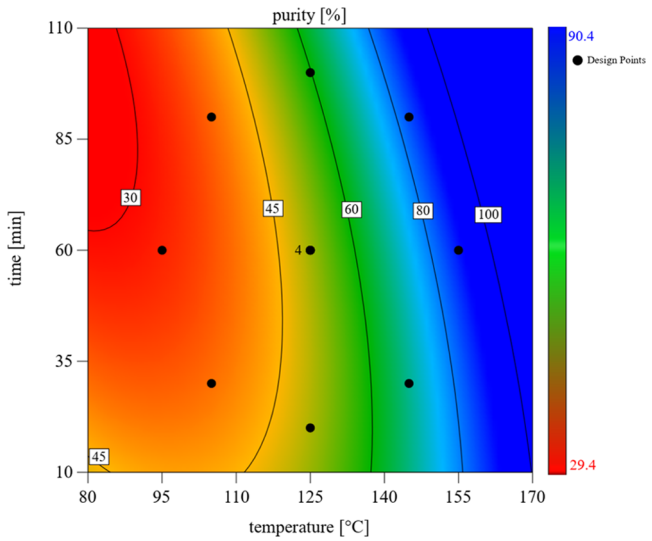


Figure 6. Purity of the extract: divided into low purity (red), medium purity (green), and high purity (blue).

and 45% (indicated by red-yellow color range) was obtained at extraction temperatures below 110 °C. The highest purity of 90% (blue) was measured after 60 min extraction at 155 °C (for measured values, see Table A1).

Monomer Composition. As an example for the monomeric composition of the separate sugars, the center point of the experimental design (Figure 1) at 125 °C and 60 min extraction time was chosen. Table 2 shows the mean analyzed masses of three separate runs for every monomer calculated in percent related to the sum of the monomer masses in the analyzed material.

Table 2. Example for Monomeric Composition of Hydrolyzed Hemicellulose Sample after Hot-Water Extraction

	glucose	galactose	xylose	arabinose	mannose
composition (%)	50.7	31.4	13.7	2.2	2.0

The results showed that glucose and galactose make up the highest proportion. The other monomers were found in descending order with xylose, arabinose, and mannose.

Hemicellulose Yield. By multiplying the purity values with the overall yield from 25 g of apple pomace, the hemicellulose yield was calculated. The highest yields could be obtained between 120 and 150 °C (Figure 7). The maximum of 5.0 wt % yield was reached at an extraction temperature of 145 °C with 30 min extraction time. By increasing the extraction time, the yield started to slightly decrease. This correlates with Cocero et al., where hemicellulose cleaving is described.[19] Oligomer cleaving takes place with a small delay. At the start of the extraction, only free sugars and a small number of cleaving products are solubilized. The molecular weight reaches its maximum when oligomers are cleaved to the degree at which

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

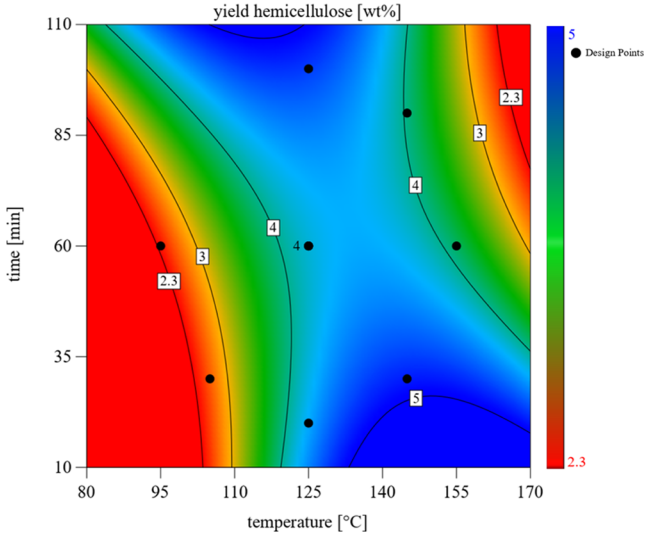


Figure 7. Hemicellulose yield from 25 g of apple pomace: divided into low yield (red), medium yield (green), and high yield (blue).

they become soluble in water. After reaching the maximum, the molecular weight decreases with time due to progressive hydrolysis of polymer chains.[19] Since bigger oligomers are more likely to be precipitated by ethanol, the hemicellulose yield starts to decrease with time because more cleaving takes place.

Influence of Extraction Conditions on Purity. In general, more severe extraction conditions resulted in a purer product at the expense of yield. Hemicellulose extraction with water starts at around 90 °C but takes place very slowly.[19] At these temperatures, side materials like proteins will be extracted along with the hemicellulose. By increasing the temperature, these side materials seem to degrade to the point where they will not precipitate by ethanol anymore. By increasing the temperature, hemicellulose cleaving becomes more rapid. Around 160 °C, hemicellulose cleaving becomes significant, resulting in a very low yield. For example, an additional extraction at 160 °C with an extraction time of 120 min was performed, resulting in an overall yield of 1%. The efficiency of extreme conditions like these is very low, and so it is not suited for industrial applications.

Hemicellulose extraction with subcritical water from wood chips may yield products with a purity of above 70%, but fibrous fruit residues will release substantial amounts of other substances. This investigation shows that at extraction temperatures below 120 °C, a large fraction of the precipitated product is not a sugar structure. Although we did not identify components of this fraction, we have shown that with increasing extraction intensity, the components are converted to molecules that are not precipitated at an alcohol concentration of 70% (v/v). The higher purity is in agreement with the lower yield of about 5% after intensified extraction, a reasonable value for our raw material, since with fiber analysis according to van Soest[26] (extended Weende analysis) a hemicellulose content of 7% is determined. The value is calculated as the difference between the fiber mass, which after amylase treatment is extracted with neutral detergent[27] and the fiber mass extracted with acid detergent.[28,29] For calculating the resource efficiency of the production process, yield is highly important. For example, with a doubling of yield, the energy demand per mass unit of product is lowered to 50%. To achieve a higher yield, it will be necessary to release glycans from the cellulose fraction (about 20%) as well. Mechano-chemical treatments followed by water extraction are currently being studied in our laboratories.[30]

Parameters of Ethanol Precipitation. Ethanol concentration and pH value are important factors that influence the raw yield and purity in precipitation.[31] An established approach to investigate the effect of varying parameters is a statistically designed experiment.[32] The chosen approach by Design-Expert 13 (Stat-Ease, Minneapolis) is shown in Table 3. This design aims at certain responses, which are the raw yield and purity of hemicelluloses. Hu et al. gave a good overview of how ethanol (and other nonsolvents) concentration affects the raw yield and purity in the precipitation process.[31]

Table 3. Statistical Independent Experimental Setups Predetermined by Design-Expert 13 to Analyze the Influence of Varying Ethanol Concentrations and pH Values in Raw Extract

run	c(EtOH) (% (v/v))	pH	raw yield (% of DM)	purity (%)
1	70	3.5	3.5	87.7
2	85	3.5	4.6	53.8
3	60	3	3.8	81.2
4	70	3.5	3.6	84.8
5	70	4.5	4.8	58.0
6	80	4	6.9	70.6
7	70	2.5	3.6	63.8
8	70	3.5	2.9	89.5
9	70	3.5	3.9	90.2
10	55	3.5	1.6	85.2
11	60	4	3.6	79.8
12	80	3	6.4	76.5

Effect of Ethanol Concentration. It has been observed that low ethanol concentrations (50% (v/v) and less) did not lead to any considerable hemicellulose precipitation. The raw yield is correlated to the ethanol concentration (high concentration equals high raw yield), which can be shown by a precipitation sequence where the pH value was fixed at 3.5, and the ethanol concentration varied from 55% (v/v) to 85% (v/v). The resulting raw yield (Figure 8a) ranged from 116.8 mg (1.6% of dry matter) to 344.7 mg (4.6% of dry matter). Sugar concentration (purity, Figure 8b) represents the second important characteristic to give a qualitative statement about the obtained hemicelluloses.

The analysis of sugar content resulted in 85% (55% (v/v) ethanol), 88% (70% (v/v) ethanol), and 54% (85% (v/v) ethanol) as determined by PSA. Example UV/Vis spectra are represented in Figure 9.

The decreasing sugar content above 70% (v/v) ethanol correlated with increasing raw yields, indicates precipitation of impurities such as proteins, e.g., which are more common in high ethanol concentrations.[33] Sugar content analyzed by ion chromatography for precipitate at pH 3.5 with 70% (v/v) ethanol resulted in 85.7%. The PSA method was found to be precise with an error of ±2% (n = 12).[34] A slightly lower value for ion chromatography may be explained by the different measuring principles. With ion chromatography, degradation products of sugar monomers that occur during hydrolysis with sulfuric acid are not included, while in the PSA method, sugar conversion to aldehydes is a prerequisite for detection.

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

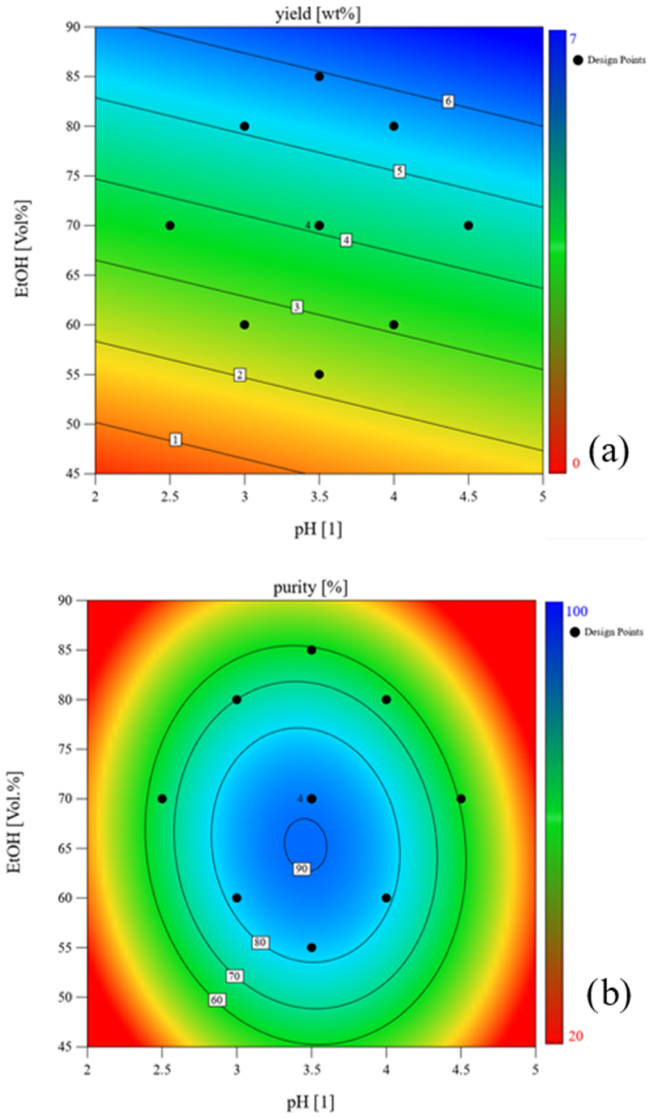


Figure 8. Raw yield (a) and purity by PSA (b) of raw extract precipitation ranging from low (red) to high (blue).

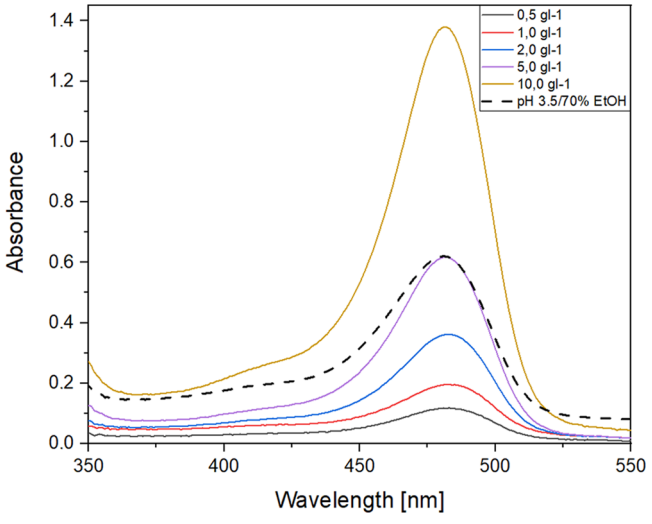


Figure 9. UV−vis spectra of a hemicelluloses sample precipitated from raw extract with 70% (v/v) ethanol at pH 3.5. Calibration spectra measured with dilutions ranging from 0.5 to 10 g/L glucose.

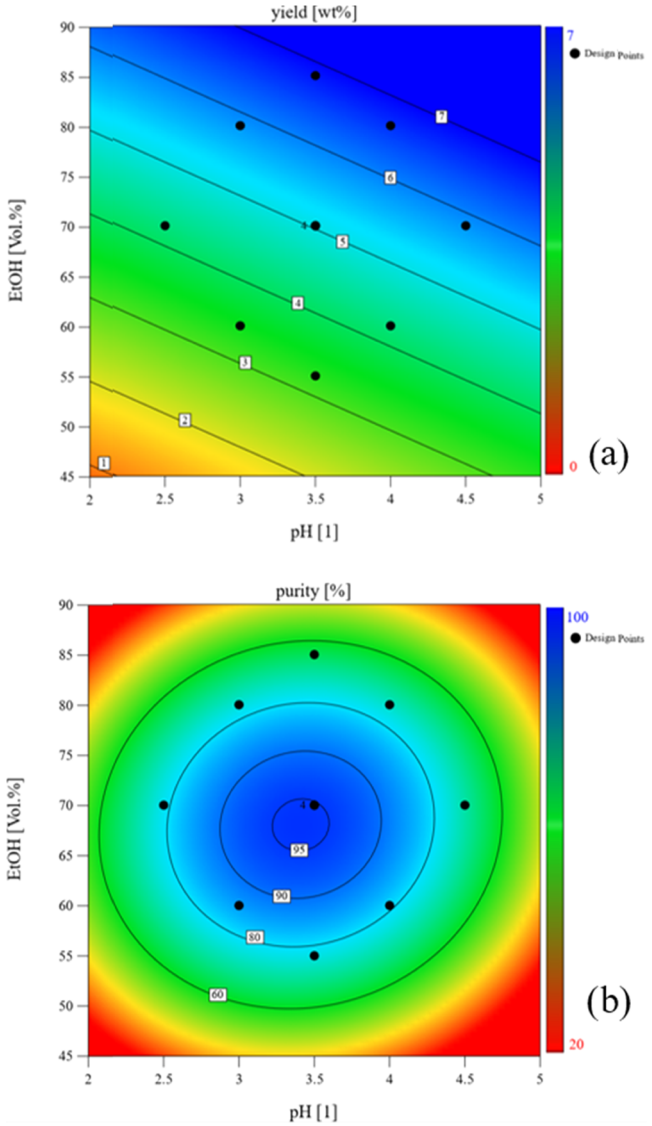


Figure 10. Raw yield (a) and purity (b) of 6x-retentate precipitation ranging from low (red) to high (blue).

Table 4. Statistical Independent Experimental Setups Predetermined by Design-Expert 13 to Analyze the Influence of Varying Ethanol Concentrations and pH Values in 6x-Retentate

run	c(EtOH) (% (v/v))	pH	raw yield (% of DM)	purity (%)
1	70	3.5	5.3	93.4
2	85	3.5	7.7	68.5
3	60	3	3.8	80.5
4	70	3.5	5.3	99.2
5	70	4.5	5.5	72.2
6	80	4	6.7	72.5
7	70	2.5	4.3	82.0
8	70	3.5	5.2	97.4
9	55	3.5	3.2	80.7
10	60	4	4.1	78.8
11	80	3	4.6	70.9
12	70	3.5	4.8	95.4

Effect of pH Value. Besides ethanol concentration, pH value is a reasonable parameter to be considered for hemicellulose

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

Table A1. Original Data for Respone Factor Overall Yield, Purity, and Hemicelluloses Yield of Hot Water Extraction[a]

run	T (°C)	t (min)	overall yield (%)	purity (%)	hemicellulose yield (%)
E001	125	60	8.14	n. d.	n.d.
E002	125	60	8.44	n. d.	n.d.
E003	125	60	8.37	n. d.	n.d.
E004	125	60	8.16	50.63	4.13
E005	105	90	7.67	41.24	3.16
E006	105	30	6.78	40.97	2.78
E007	145	90	4.70	83.67	3.93
E008	145	30	7.42	66.92	4.96
E009	125	100	8.24	57.99	4.78
E010	125	20	8.36	49.84	4.16
E011	155	60	4.27	90.42	3.86
E012	95	60	7.82	29.41	2.30

[a]For the center point, purity was determined for a single replicate (n.d. denotes not determined). In order to illustrate the measured levels for purity and hemicellulose yield (derived from overall yield and purity) in the form of a contour plot, model calculation is necessary. This model calculation was based on the assumption that repeated purity analysis at the center point yields the same result. The resulting models (Tables A3 and AA4) have the sole function to provide a graphical overview of measured purities in the form of a contour plot.

precipitation. Hydroxyl groups as functional groups in the polysaccharide chains are affected by pH changes.[35] Water molecules bind to hydroxyl groups by weak interactions, forming a hydration layer which makes the polymer soluble in aqueous solutions. These hydration layers can either be broken by a pH change or by adding a reagent, which competes for water with the polysaccharides. Hemicelluloses may contain carboxyl groups, which become protonated between pH 3 and 5. Protonation has a profound influence on the solubility of the sugar structure, since the negatively charged molecule becomes electrically neutral. As competing reagents, salts like NaCl or $(NH_4)_2SO_4$ would be suitable.[36] To eliminate the salt from precipitates, an additional processing step would be necessary.[31,37] Therefore, the less time-consuming approach by changing pH value was investigated. The performed hot-water extraction resulted in a glycan solution with a pH value around pH 3.5−4. The statistical design covered the pH range between 2.5 and 4.5 with a step-size of 0.5 units. The influence of pH on raw yield was not significant, but ethanol concentration had a large impact. For a fixed ethanol concentration, the pH value shows a marginal effect on purity and a low impact on raw yield. At 70% (v/v) ethanol, the raw yield ranged from 268.2 mg (3.6% of dry matter) at pH 2.5 to 362.7 mg at pH 4.5 (4.8% of dry matter). Both samples are at

Table A2. ANOVA Data (Quadratic Model) and Fit Statistics for Response Factor Overall Yield by Hot Water Extraction after Excluding the Outlier at 105 °C/90 min

source	sum of squares	df	mean square	F-value	p-value	
model	22.24	5	4.45	171.40	<0.0001	significant
A-temp	*8.77*	*1*	*8.77*	*337.84*	*<0.0001*	
B-Zeit	*6.519E-003*	*1*	*6.519E-003*	*0.25*	*0.6375*	
AB	*5.10*	*1*	*5.10*	*196.59*	*<0.0001*	
A^2	*7.57*	*1*	*7.57*	*291.77*	*<0.0001*	
B^2	*1.423E-003*	*1*	*1.423E-003*	*0.055*	*0.8241*	
Residual	0.13	*5*	0.026			
lack of fit	*0.061*	*2*	*0.030*	*1.31*	*0.3896*	not significant
pure error	*0.069*	*3*	*0.023*			
cor total	22.37	10				
std. dev.	0.16		R^2		0.9942	
mean	7.34		adj R^2		0.9884	
C.V.%	2.20		pred R^2		0.9543	
PRESS	1.02		adeq precision		33.937	

Table A3. ANOVA Data (Quadratic Model) and Fit Statistics for Response Factor Purity of Hemicelluloses by Hot Water Extraction

source	sum of squares	df	mean square	F-value	p-value	
model	3340.44	5	668.09	133.96	<0.0001	significant
A-temp	3007.46	1	3007.46	603.06	<0.0001	
B-Zeit	102.98	1	102.98	20.65	0.0039	
AB	67.85	1	67.85	13.61	0.0102	
A^2	153.19	1	153.19	30.72	0.0015	
B^2	29.40	1	29.40	5.89	0.0513	
residual	29.92	6	4.99			
lack of fit	29.92	3	9.97			
pure error	0.0000	3	0.0000			
cor total	3370.36	11				
std. dev.	2.23		R^2		0.9911	
mean	55.25		adj R^2		0.9837	
C.V.%	4.04		pred R^2		0.9313	
			adeq precision		35.7360	

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

Table A4. ANOVA Data (Quadratic Model) and Fit Statistics for Response Factor Hemicelluloses Yield of Hot Water Extraction (Calculated From Overall Yield and Purity) after Excluding the Outlier at 105 °C/90 min

source	sum of squares	df	mean square	*F*-value	*p*-value	
model	6.03	5	1.21	75.98	0.0001	significant
A-temp	*1.65*	*1*	*1.65*	*103.98*	*0.0002*	
B-Zeit	*0.13*	*1*	*0.13*	*8.47*	*0.0334*	
AB	*0.87*	*1*	*0.87*	*54.74*	*0.0007*	
A^2	*1.66*	*1*	*1.66*	*104.58*	*0.0002*	
B^2	*0.18*	*1*	*0.18*	*11.26*	*0.0202*	
residual	*0.079*	*5*	*0.016*			
lack of fit	*0.062*	*2*	*0.031*	*5.21*	*0.1057*	not significant
pure error	*0.018*	*3*	*5.916E-003*			
cor total	6.11	10				
std. dev.	0.13		R^2		0.9870	
mean	3.96		adj R^2		0.9740	
C.V.%	3.18		pred R^2		0.8552	
PRESS	0.88		adeq precision		26.794	

Table A5. ANOVA Data (Quadratic Model) and Fit Statistics for Response Factor Purity of Raw Extract Precipitation

source	sum of squares	df	mean square	*F*-value	*p*-value	
model	1551.42	5	310.28	11.56	0.0049	significant
A-pH	29.77	1	29.77	1.11	0.3329	
B-EtOH	437.76	1	437.76	16.30	0.0068	
AB	5.06	1	5.06	0.1885	0.6793	
A^2	955.64	1	955.64	35.59	0.0010	
B^2	419.43	1	419.43	15.62	0.0075	
residual	161.11	6	26.85			
lack of fit	143.70	3	47.90	8.25	0.0583	not significant
pure error	17.41	3	5.80			
cor total	1712.53	11				
std. dev.	5.18		R^2	0.9059		
mean	76.76		adjusted R^2	0.8275		
C.V.%	6.75		predicted R^2	0.4432		
			ad precision	8.1376		

Table A6. ANOVA Data (Quadratic Model) and Fit Statistics for Response Factor Purity of Retentate Precipitation

source	sum of squares	df	mean square	*F*-value	*p*-value	
model	1214.06	5	242.81	11.23	0.0053	significant
A-pH	32.34	1	32.34	1.50	0.2671	
B-EtOH	137.60	1	137.60	6.37	0.0451	
AB	2.72	1	2.72	0.1259	0.7348	
A^2	537.35	1	537.35	24.86	0.0025	
B^2	825.39	1	825.39	38.18	0.0008	
residual	129.70	6	21.62			
lack of fit	110.87	3	36.96	5.89	0.0897	not significant
pure error	18	83	3	6.28		
cor total	1343.76	11				
std. dev.	4.65		R^2	0.9035		
mean	82.62		adjusted R^2	0.8230		
C.V.%	5.63		predicted R^2	0.3604		
			ad precision	9.1329		

low purity values of 58.0% (pH 4.5) and 63.8% (pH 2.5), which are not satisfactory.

At 60% (v/v) and 80% (v/v) ethanol, the raw yield and purity are almost identical for different pH values. The most significant difference was found to be at 70% (v/v) ethanol at pH 3.5. The raw yield is lower compared to pH 4.5, but the purity increased to 88%, as written above. The ANOVA data in Table A5 show that the terms B, A^2, and B^2 (A = pH value, B = ethanol concentration) are significant (p-value $\leq$ 0.05), whereas terms A and AB are not significant. The terms in descending order $A^2 > B > B^2$ affect the precipitation process. For these parameters 69% (v/v) ethanol and pH 3.5 were determined by numerical optimization (maximizing purity within 4 to 7 wt % yield) to result in the most satisfactory hemicellulose precipitates with a yield of 4 wt % and 88% purity (desirability = 0.468).

Retentate Precipitation. The precipitation process requires adding four times the volume of glycan solution as ethanol. At

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

Table A7. ANOVA Data (Linear Model) and Fit Statistics for Response Factor Yield of Raw Extract Precipitation

source	sum of squares	df	mean square	*F*-value	*p*-value	
model	13.33	2	6.67	6.34	0.0192	significant
A-pH	0.6075	1	0.6075	0.5775	0.4667	
B-EtOH	12.72	1	12.72	12.10	0.0070	
residual	9.47	9	1.05			
lack of fit	8.94	6	1.49	8.47	0.0538	not significant
pure error	0.5275	3	0.1758			
cor total	22.80	11				
std. dev.	1.03		R^2	0.5847		
mean	4.10		adjusted R^2	0.4925		
C.V.%	25.02		predicted R^2	0.2173		
			ad precision	7.1575		

Table A8. ANOVA Data (Linear Model) and Fit Statistics for Response Factor Yield of Retentate Precipitation

source	sum of squares	df	mean square	*F*-value	*p*-value	
model	14.04	2	7.02	22.82	0.0003	significant
A-pH	1.92	1	1.92	6.24	0.0340	
B-EtOH	12.12	1	12.12	39.40	0.0001	
residual	2.77	9	0.3077			
lack of fit	2.60	6	0.4331	7.64	0.0618	not significant
pure error	0.1700	3	0.0567			
cor total	16.81	11				
std. dev.	0.5547		R^2	0.8353		
mean	5.04		adjusted R^2	0.7987		
C.V.%	11.00		predicted R^2	0.6632		
			ad precision	12.9172		

the laboratory scale, a total liquid volume of 500 mL per precipitation is processed. When upscaling the process to the pilot plant, a total volume of roughly 350 L aqueous ethanol mixture remains after separating the precipitate from the liquid. To recycle the used ethanol, a rectification is integrated in the pilot plant process, which needs to run after every precipitation process. Separating ethanol and water by rectification is an energy-consuming process step that we wish to reduce. For achieving this, the total process volume needs to be lowered, starting with the glycan solution. The glycan solution was concentrated by ultrafiltration with a ceramic hollow-fiber membrane (cutoff 1 kDa). Eventually, we could precipitate hemicelluloses from a six times smaller volume, which lowers the need for ethanol by 83%.

To get a comparable amount of hemicellulose precipitate, 16.7 mL of glycan solution (equivalent to 100 mL raw glycan solution) was precipitated (Figure 10) by the same procedure as for the raw extract described above.

The influence of pH and ethanol concentration on yield and purity was similar for the concentrated extract (retentate) compared to the raw extract. Yields at pH 3.5 were 237.8 mg, 395.6 mg, and 573.8 mg at ethanol concentrations (v/v) of 55, 70, and 85%, respectively (Table 4). Compared to the raw extract, yield increased to 204, 150, and 166% at ethanol concentrations of 55, 70, and 85%, respectively. The purity was very similar to raw extract precipitates for ethanol concentrations of 60 and 80%. Likewise, pH adjustment did not improve purity for these ethanol concentrations. At an ethanol concentration of 70%, pH had a similar influence on purity compared to raw extract precipitates.

The parameters 70%(v/v) ethanol and pH 3.5 are promising since a purity of 96.4% (PSA; 86.1% ion chromatography) and a raw yield of 395.6 mg are achieved. The numerical optimization predicted a maximum purity of 96% with 4.7 wt % yield as highest yield with this purity (desirability = 0.786).

Influence of Precipitation Parameters on Quality and Energy Demand of the Hemicellulose Product. Hemicelluloses precipitation from raw extracts and concentrated retentates both showed similar behaviors comparing raw yield and purity. Precipitation with low ethanol concentrations (55% (v/v) and less) resulted in lowest yields with a purity of roughly 80−85%, whereas using high ethanol concentrations (85% (v/v)) led to more raw yield but low purity of about 55−60%. The investigation of pH value adjustment led to the conclusion that the effect on precipitation is not as significant as changing ethanol concentration. The precipitation process with 70% (v/v) ethanol at pH 3.5 was found to be most suitable for our glycan extracts to achieve proper raw yields and satisfactory purity of the hemicelluloses.

The concentration of raw extracts by ultrafiltration has a slightly positive effect on product yield and purity. The impact of ultrafiltration on resource efficiency may even be more important. The energy demand for the extraction process at the pilot scale is mainly governed by the process for alcohol recovery (not shown). The data of this paper show that the alcohol demand can be reduced to 1/6 with ultrafiltration of the crude extract. Currently, the lab-scale filtration process requires nearly the same energy amount as alcohol recovery.

Filtration technologies counteracting the continuous increase of membrane resistance, which maintain a large cross-flow velocity at a low trans-membrane pressure, will have a large impact on the energy demand. Furthermore, it will be important to quantify the energy savings which result from the upscaling of ultrafiltration operations.

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447

Although glycans from fruit residues require alcohol precipitation, which is not required for the production of chemical pulp and starch, they represent a better building block for resins in which structural diversity is an advantage. They provide backbones with a large number of short side chains, different monomers with the possibility to selectively substitute, and resistance to starch-degrading enzymes.

CONCLUSIONS

- Aqueous extracts of hemicelluloses from fruit residues have been demonstrated to provide glycan products with a purity of 90% in a two-step process with ultrafiltration and alcohol precipitation. Both processes are scalable to an industrial level.
- Ultrafiltration serves a dual purpose. It strongly reduces the required amount of alcohol, and it improves the purity of the precipitate.
- When cellulose is included as a glycan source, the yield will be in the range of the production of pulp or potato starch.
- Because of non-wood raw materials, alkali and bleaching agents are not required.

APPENDIX

Raw data for yield and purity of hot-water extraction, results from ANOVA and fit statistics can be found in Tables A1−A8.

AUTHOR INFORMATION

Corresponding Author

Stefan Hanstein − *Fraunhofer Research Institution for Materials Recycling and Resource Strategies IKWS, 63755 Alzenau, Germany;* orcid.org/0000-0001-6636-0904; Email: stefan.hanstein@iwks.fraunhofer.de

Authors

Marius Wolf − *Fraunhofer Research Institution for Materials Recycling and Resource Strategies IKWS, 63755 Alzenau, Germany;* orcid.org/0000-0002-6176-5117

Frederik Berger − *Fraunhofer Research Institution for Materials Recycling and Resource Strategies IKWS, 63755 Alzenau, Germany;* orcid.org/0000-0003-1404-391X

Anke Weidenkaff − *Fraunhofer Research Institution for Materials Recycling and Resource Strategies IKWS, 63755 Alzenau, Germany;* orcid.org/0000-0002-7021-1765

Hans-Ulrich Endreß − *Herbstreith & Fox GmbH & Co. KG Pektin-Fabriken, 75305 Neuenbürg, Germany*

Arne Michael Oestreich − *University of Applied Sciences Giessen Friedberg, 35390 Gießen, Germany;* orcid.org/0000-0002-3054-7440

Mehrdad Ebrahimi − *University of Applied Sciences Giessen Friedberg, 35390 Gießen, Germany;* orcid.org/0000-0003-4737-8257

Peter Czermak − *University of Applied Sciences Giessen Friedberg, 35390 Gießen, Germany;* orcid.org/0000-0001-7485-500X

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c06055

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The project was funded by the German Federal Ministry of Education and Research Grant No. 031B0905A. Experiments with different alcohol concentrations and with extracts after ultrafiltration are part of the Ph.D. thesis of Marius Wolf. Funding of the Ph.D. project by Herbstreith & Fox GmbH & Co. KG Pektin-Fabriken is gratefully acknowledged. This investigation would not have been possible without the incentive of the HyperBioCoat project, which has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 720736 (https://hyperbiocoat.eu; https://cordis.europa.eu/project/id/720736/reporting). During this project, extraction with subcritical water at pilot scale was initiated at Fraunhofer Research Institution IWKS, and depectinized apple pomace was identified as a promising raw material source. Funding of safety infrastructure around the pilot plant (ATEX compliance) and validation of pilot-scale extraction from apple pomace is gratefully acknowledged.

ABBREVIATIONS USED

CFV, cross-flow velocity; EtOH, ethanol; HPIC-PAD, high-performance-ion-chromatography-pulsed amperometric detector; PSA, phenol sulfuric acid assay; TMP, trans-membrane pressure; UV/Vis, ultraviolet/visible

REFERENCES

(1) Nova Institute. Bio-based Building Blocks and Polymers - Global Capacities, Production and Trends 2020−2025: Short Version, https://renewable-carbon.eu/publications (accessed June 23, 2021).

(2) Poth, U. *Polyester and Alkyd Resins: Technical Basics and Applications*; Vincentz Network, 2020.

(3) Hansen, N. M. L.; Plackett, D. Sustainable films and coatings from hemicelluloses: A review. *Biomacromolecules* **2008**, *9*, 1493−1505.

(4) Hubbe, M. Prospects for maintaining strength of paper and paperboard products while using less forest resources: A review. *BioResources* **2013**, *9*, 1634−1763.

(5) Xu, G.-B.; Kong, W.-Q.; Liu, C.-F.; Sun, R.-C.; Ren, J.-L. Synthesis and characteristic of xylan-grafted-polyacrylamide and application for improving pulp properties. *Materials* **2017**, *10*, No. 971.

(6) Ryberg, Y. Z.; Edlund, U.; Albertsson, A.-C. Innovative approaches for converting a wood hydrolysate to high-quality barrier coatings. *ACS Appl. Mater. Interfaces* **2013**, *5*, 7748−7757.

(7) Ghosh, D.; Tanner, J.; Lavoie, J.-M.; Garnier, G.; Patti, A. F. Integrated approach for hemicellulose extraction from forest residue. *BioResources* **2021**, *16*, 2524−2547.

(8) Joubert, A. J.; Chimphango, A. F. A.; Goergens, J. F. Effect of integrating xylan extraction from *E. grandis* into the Kraft pulping process on pulp yield and chemical balance. *BioResources* **2016**, *11*, 2417−2437.

(9) Grigoray, O.; Järnström, J.; Heikkilä, E.; Fardim, P.; Heinze, T. Modification of pine pulp during oxygen delignification by xylan self-assembly. *Carbohydr. Polym.* **2014**, *112*, 308−315.

(10) Shao, H.; Hu, Y.; Sun, H.; Yang, B.; Fan, B.; Zhang, H. Response surface optimization of alkali extraction and characterization of poplar hemicellulose. *BioResources* **2019**, *14*, 3844−3859.

(11) Stoklosa, R. J.; Hodge, D. B. Extraction, recovery, and characterization of hardwood and grass hemicelluloses for integration into biorefining processes. *Ind. Eng. Chem. Res.* **2012**, *51*, 11045−11053.

(12) Zhang, J.; Cai, Di.; Qin, Y.; Liu, D.; Zhao, X. High value-added monomer chemicals and functional bio-based materials derived from

polymeric components of lignocellulose by organosolv fractionation. *Biofuels, Bioprod. Biorefin.* **2020**, *14*, 371−401.
(13) Adams, M. F.; Ettling, B. V. Larch Arabinogalactan. In *Industrial Gums*, 2nd ed.; Whistler, R. L., Ed.; Academic Press: New York, 1973; Vol. *46*, pp 415−427.
(14) Martínez-Abad, A.; Giummarella, N.; Lawoko, M.; Vilaplana, F. Differences in extractability under subcritical water reveal interconnected hemicellulose and lignin recalcitrance in birch hardwoods. *Green Chem.* **2018**, *20*, 2534−2546.
(15) Andérez Fernández, M.; Rissanen, J.; Nebreda, A. P.; Xu, C.; Willfor, S.; Garcia Serna, J.; Salmi, T.; Grenman, H. Hemicelluloses from stone pine, holm oak, and Norway spruce with subcritical water extraction - comparative study with characterization and kinetics. *J. Supercrit. Fluids* **2018**, *133*, 647−657.
(16) Gallina, G.; Cabeza, A.; Grenman, H.; Biasi, P.; Garcia-Serna, J.; Salmi, T. Hemicellulose extraction by hot pressurized water pretreatment at 160 degrees C for 10 different woods: Yield and molecular weight. *J. Supercrit. Fluids* **2018**, *133*, 716−725.
(17) Yedro, F. M.; Cantero, D. A.; Pascual, M.; Garcia-Serna, J.; Cocero, M. J. Hydrothermal fractionation of woody biomass: Lignin effect on sugars recovery. *Bioresour. Technol.* **2015**, *191*, 124−132.
(18) Karlsson, M.; Giummarella, N.; Linden, P. A.; Lawoko, M. Toward a Consolidated Lignin Biorefinery: Preserving the Lignin Structure through Additive-Free Protection Strategies. *ChemSusChem* **2020**, *13*, 4666−4677.
(19) Cocero, M. J.; Cabeza, Á.; Abad, N.; Adamovic, T.; Vaquerizo, L.; Martínez, C. M.; Pazo-Cepeda, M. V. Understanding biomass fractionation in subcritical & supercritical water. *J. Supercrit. Fluids* **2018**, *133*, 550−565.
(20) Willför, S.; Pranovich, A.; Tamminen, T.; Puls, J.; Laine, C.; Suurnäkki, A.; Saake, B.; Uotila, K.; Simolin, H.; Hemming, J.; Holmbom, B. Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides−A comparison between different hydrolysis and subsequent chromatographic analytical techniques. *Ind. Crops Prod.* **2009**, *29*, 571−580.
(21) Saeman, J. F.; Moore, W. E.; Mitchell, R. L.; Millett, M. A. Techniques for the determination of pulp constituents by quantitiative paper chromatography. *Tappi J.* **1954**, *37*, 336−343.
(22) DuBois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.
(23) Chaplin, M. F.; Kennedy, J. F. *Carbohydrate Analysis. A Practical Approach*; IRL Press: Washington DC, 1986; Vol. *143*.
(24) Scherz, H.; Bonn, G. *Analytical Chemistry of Carbohydrates*; G. Thieme Verlag: New York, 1998.
(25) Viel, M.; Collet, F.; Lanos, C. Chemical and multi-physical characterization of agro-resources' by-product as a possible raw building material. *Ind. Crops. Prod.* **2018**, *120*, 214−237.
(26) Soest, P. J. V. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. AOAC* **1963**, *46*, 829−835.
(27) AOAC International. Fiber/amylase-treated Neutral Detergent Fiber in Feeds: Rockville, MD (Method 2002.04). http://www.eoma.aoac.org/ (accessed Dec 21, 2021).
(28) International Organization for Standardization (ISO). Animal Feeding Stuffs—Determination of Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) Contents: Geneva, Switzerland, 2008 (ISO 13906:2008). https://www.iso.org/standard/43032.html (accessed Dec 21, 2021).
(29) AOAC International. Fiber (Acid Detergent) and Lignin in Animal Feed. In: Official Methods of Analysis, 17th Edition. Association of Official Analytical Chemists: Arlington, VA, 2000 (Method 973.18). http://www.eoma.aoac.org/ (accessed Dec 21, 2021).
(30) Käldström, M.; Meine, N.; Farès, C.; Schüth, F.; Rinaldi, R. Deciphering 'water-soluble lignocellulose' obtained by mechanocatalysis: new insights into the chemical processes leading to deep depolymerization. *Green Chem.* **2014**, *16*, 3528−3538.
(31) Hu, X.; Goff, H. D. Fractionation of polysaccharides by gradient non-solvent precipitation: A review. *Trends Food Sci. Technol.* **2018**, *81*, 108−115.
(32) Samavati, V. Polysaccharide extraction from *Abelmoschus esculentus*: optimization by response surface methodology. *Carbohydr. Polym.* **2013**, *95*, 588−597.
(33) Huang, Q.-L.; Siu, K.-C.; Wang, W.-Q.; Cheung, Y.-C.; Wu, J.-Y. Fractionation, characterization and antioxidant activity of exopolysaccharides from fermentation broth of a *Cordyceps sinensis* fungus. *Process Biochem.* **2013**, *48*, 380−386.
(34) Nielsen, S. *Food Analysis*; Food Science Text SeriesSpringer US: New York, 2012.
(35) Hedayati, S.; Shahidi, F.; Koocheki, A.; Farahnaky, A.; Majzoobi, M. Physical properties of pregelatinized and granular cold water swelling maize starches at different pH values. *Int. J. Biol. Macromol.* **2016**, *91*, 730−735.
(36) Perger, H. Untersuchungen über das Aussalzen der Polysaccharide und über den Verlauf der Säurehydrolyse der Stärke. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere* **1922**, *196*, 92−112.
(37) Gonzaga, M. L. C.; Ricardo, N. M.; Heatley, F.; Soares, S. de A. Isolation and characterization of polysaccharides from *Agaricus blazei* Murill. *Carbohydr. Polym.* **2005**, *60*, 43−49.

https://doi.org/10.1021/acsomega.1c06055
ACS Omega 2022, 7, 13436−13447