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Semi-bleached paper and fermentation products from a larch biorefinery

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ABSTRACT: This study was focused on the products from a larch biorefinery, specifically bleached paper and different fermentation products. Siberian larch (*Larix sibirica* Lebed.) wood chips were treated with water in a pre-extraction (PE) stage. The larch extract was removed by drainage and fermented into different products. Eight different bacteria strains were tested. The extracted wood chips were mildly washed before kraft pulping with polysulfide (PS) and anthraquinone (AQ). The PE-PSAQ pulps were bleached to about 80% brightness. Laboratory paper sheets were made and tested for different paper properties, and a conventional larch kraft pulp was also prepared as reference. The larch PE-PSAQ paper and the larch kraft paper had similar properties. The removal of a significant amount of hemicelluloses from the wood chips before pulping was not a detriment to the paper properties.

Application: Combining papermaking pulp production with production of value-added products from the extracted hemicellulose stream resulted in a promising biorefinery concept.

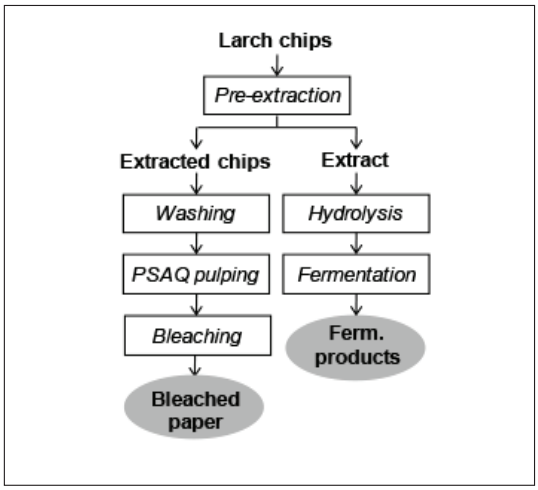
A biorefinery facility integrates biomass conversion processes and equipment to produce fuels, power, and value-added chemicals from biomass [1]. In a forest biorefinery, wood or forest biomass is converted to different products. The idea is to efficiently use all biomass raw materials and convert them into traditional forest products, such as paper and board products, as well as biofuels and chemicals [2–4]. A wide variety of products could be produced through different routes, thereby increasing revenue. For the traditional paper industry in the temperate zone, the biorefinery concept could be the solution to finding new ways to remain competitive and meet the big challenges in the market. In one of these biorefinery concepts, the hemicelluloses are extracted before kraft pulping and then used for manufacture of higher value-added products by microbial fermentation [5].

There are large resources of larch in Russia and North America [6]. Larch has a high amount of water-soluble arabinogalactan [7,8], which is an advantage in using larch wood as raw material for a water pre-extraction (PE) process. The hemicellulose-rich larch extract is suitable for fermentation, while the extracted wood chips could go through a washing stage for pulping and papermaking. The pulping additives polysulfide (PS) and anthraquinone (AQ) can compensate for the yield loss due to PE. When PS and AQ are used together in alkaline pulping, the the synergetic yield increase of PSAQ pulp is 0.5%–1.5% [9–12]. Paper made from larch kraft pulps has high tear strength, but other strength properties are somewhat weaker than those of paper made from pine and spruce pulps [13–15].

Most industrial microorganisms ferment only mono- and disaccharides; therefore, the extract has to be hydrolyzed before fermentation. The choice of microorganism for producing target substances is determined by sugar composition of the hydrolysate and substrate specificity of a strain. Also important is a microorganism's tolerance to hydrolysis byproducts, such as acetic and formic acids, lignin, furfural, and hydroxymethylfurfural, which inhibit fermentation at high concentrations. Byproducts are toxic to most industrial microorganisms, but the concentration of inhibitors depends on temperature and duration of the hydrolysis and the source of plant raw material [16]. The problem can be solved by detoxification of acid hydrolysates or by searching for microorganisms tolerant to inhibitors.

In bacterial fermentation, *Clostridium* species producing acetone, butanol, ethanol, acetate, and butyrate ferment both hexoses and pentoses efficiently. Acid hydrolysates from corn stover, sun flower shells, and hemp waste mixed with flour and molasses were used successfully in large-scale clostridial fermentation [16,17]. Some species of *Lactobacillus* are tolerant of the toxic components of acid hydrolysates, as well as to increased concentrations of alcohols and acids [18–21]. Various *Lactobacillus* species are able to use a wide spectrum of monosugars, including those present in larch hemicellulose.

Earlier experiments by our research group provided the proof of concept for this larch biorefinery (i.e., pre-extraction PSAQ pulping of Siberian larch wood chips). With complete washing of the extracted chips, a 4.0% PS charge can compensate the yield loss caused by the pre-extraction [22]. The optimal conditions for the pre-extraction were determined to be 150°C for 90 min [5]. The fermentation of the larch extract with *Bacillus coagulans* into lactic acid was investigated with good results [5]. The PE-PSAQ process was optimized to make it more practical [23]. A mild wash of the extracted chips before the cooking stage was shown to be sufficient to remove the dissolved carbohydrates, and a PS charge of 2.0% significantly compensated the yield loss due to the pre-extraction. Such a larch biorefinery (**Fig. 1**) could have both a technical



1. Process scheme of a larch biorefinery producing semi-bleached paper and different fermentation products.

and an economical potential [24].

Our aim was to investigate the products produced from the larch biorefinery. The PE-PSAQ pulps were bleached and laboratory paper sheets were produced and tested, and the larch water extract was fermented into different fermentation products.

EXPERIMENTAL

Pre-treatment and pulping

Siberian larch (*Larix sibirica* Lebed.) mill chips from Russia were pre-extracted and washed before pulping with PS and AQ. The laboratory water PE trials were conducted in a hot-air-bath cooking system containing six rotating autoclaves (2.5 L each). After PE the extract was drained and the chips were mildly washed with fresh water. The wash water was removed by centrifugation and was not added to the extract. The pulping trials were performed in the same digester as the PE trials. **Table I** presents information on the PE and pulping experiments, which were published earlier [23].

Bleaching

Three larch pulps—a kraft pulp and two PE-PSAQ pulps (PS charge 0.5% and 2.0%)—were bleached according to the bleaching sequence O-D0-Ep-D1-P. **Table II** presents the conditions for each bleaching stage. Oxygen delignification was conducted in the hot-air-bath cooking system and the rest of the bleaching stages were performed with plastic bags and water baths. The chloride and peroxide concentrations were determined by iodometric titration. Kappa number (SCAN-C 1:00 "Chemical pulp – Kappa number") and pH at room temperature were determined after most bleaching stages. The ISO brightness (ISO 2470 "Measurement of diffuse blue reflectance factor") was determined after the D1 stage. The properties of the bleached and unbeaten pulp were analyzed using a FiberLab device (Metso Automation; Kajaani, Finland). After bleaching, the pulps were beaten with a PFI mill at 2000 revolutions.

PE and Pulping	Kraft	PS0.5	PS2.0
Pre-extraction			
Wood, g o.d.	-	300	300
L/W	-	3.5	3.5
Extraction temperature, °C	-	150	150
Heat-up time, min	-	60	60
Extraction time, min	-	90	90
Dissolved wood collected, %	-	7.4	7.4
Washing			
L/W	-	3.5	3.5
Washing temperature, °C	-	80	80
Washing time, min	-	60	60
Dissolved wood collected, %	-	4.0	4.0
Cooking			
L/W	3.5	3.5	3.5
Cooking temperature, °C	167	167	167
Heat-up time, min	60	60	60
H-factor, h	1650	1250	1250
Effective alkali (EA) charge, %	23	20	20
Polysulfide charge, %	-	0.5	2.0
Anthraquinone charge, %	-	0.1	0.1
White liquor EA, g NaOH/L	125.6	125.5	125.5
White liquor sulfidity, %	37.6	38.0	38.0
PS liquor EA, g NaOH/L	-	123.0	119.2
PS liquor sulfidity, %	-	38.1	42.0
NaOH addition, g	-	1.0	4.1
Pulp yield, %	40.4	37.2	38.4
Kappa number	26.1	26.3	25.9

I. Pre-extraction and pulping information.

Papermaking and testing

The beaten pulps were diluted with water to consistency of 2 g/L and the Schopper Riegler (SR) numbers were determined (ISO 5267-1 "Determination of drainability—Part 1: Schopper-Riegler method"). The pulp suspensions were further diluted and laboratory paper sheets were prepared using a sheet former (ISO 5269-1 "Preparation of laboratory sheets for physical testing—Part 1: Conventional sheet-former method"). The paper sheets were wet pressed and dried on drying drums at 65°C for 2 h and conditioned at certain humidity and temperature before testing (ISO 187 "Standard atmosphere for conditioning and testing and procedure for monitoring the atmosphere and conditioning of samples"). The following paper properties were determined: basis weight, density, and bulk (ISO 534 "Determination of thickness, density and spe-

Bleaching Sequences		Concistency %	Charge %	Temperature C	Time min
O	Oxygen delignification (6 bar)	10	NaOH 2.5% Mg 0.5%	95	90
D0	Chlorine dioxide	10	ClO_2 3.0% H_2SO_4 0.65%	60	60
EP	Alkaline extraction with hydrogen peroxide	10	NaOH 1.0% H_2O_2 0.9%	85	90
D1	Chlorine dioxide	10	ClO_2 0.7% H_2SO_4 0.4%	60	60
P	Peroxide	10	NaOH 1.0% Mg 0.05% H_2O_2 0.8%	80	180

II. Description and conditions for bleaching stages.

cific volume"); roughness (SCAN-P 21:67 "Determination of roughness/smoothness [air leak methods]—Part 2: Bendtsen method"); air permeability (SCAN-P 60:87 "Determination of air permeance [medium range]—Part 3: Bendtsen method"); brightness (ISO 2470); opacity (ISO 2471 "Determination of opacity [paper backing]: Diffuse reflectance method"); light scattering and absorption (ISO 9416 "Determination of light scattering and absorption coefficients [using Kubelka-Munk theory]"); tensile strength (ISO 1924-2 "Determination of tensile properties—Part 2: Constant rate of elongation method [20 mm/min]"); tear strength (ISO 1974 "Determination of tearing resistance— Elmendorf method"); and zero span (TAPPI T 279 pm-99 "Effective fiber length index by zero/short-span tensile testing").

Fermentation

The larch water extract was acid hydrolyzed before fermentation. The pH of the extract was adjusted to 1 with 7 g/L sulfuric acid and the extract was then hydrolyzed in an autoclave at 121°C for 60 min (20-min heat-up). The insoluble lignin was filtered from the hydrolyzed extract, which was neutralized to pH 7 with 36 g/L calcium hydroxide, and the gypsum was removed through filtration.

Different bacteria strains were tested in the fermentation trials. *Lactobacillus brevis* ATCC367 and ATCC8287 and *Clostridium acetobutylicum* MSU 6 and MSU 7 [25] were obtained from the collection of Lononosov Moscow State University. *C. saccharobutylicum* VKPM B-10183 [26], *C. acetobutylicum* VKPM B-4786, and *B. coagulans* VKPM B-10468 were ordered from VKPM (Russian State Collection of Industrial Microorganisms [VKPM]). *L. plantarum* DSM 20314 strain was obtained from Aalto University, Finland.

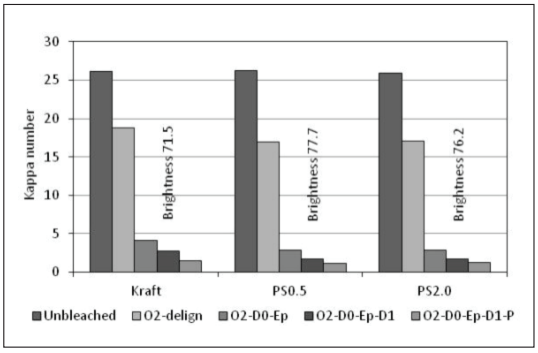
Clostridium strains were cultivated at 37°C in anaerobic flasks in the media composed of 5 g/L yeast extract, 3 g/L ammonium acetate, 1 g/L monopotassium phosphate (KH_2PO_4), 1 g/L magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_20$), 0.8 g/L K_2HPO_4, 0.5 g/L cysteine-hydrochloride, 0.05 g/L ferrous sulfate heptahydrate, and hydrolyzed larch extract (up to 1 L). *Lactobacillus* and *Bacillus* strains were grown at 30°C semi-anaerobically (without agitation in closed vials filled with the agar media) [21] in the MRS media composed of 10 g/L casein peptone, 10 g/L meat extract, 5 g/L yeast extract, 1 g/L polysorbate 80, 2 g/L K_2HPO_4, 5 g/L sodium acetate, 2 g/L ammonium citrate, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.05 g/L manganese sulfate monohydrate, and hydrolyzed larch extract (up to 1 L). Kanamycin (12.5 µg/mL) was added in case of *L. brevis* to avoid contamination of the *L. brevis* culture.

The concentrations of butanol, ethanol, and acetic and butyric acids were measured by a gas chromatograph (GC-2010, Shimadzu Corporation; Kyoto, Japan), equipped with a Phase-Stabilwax-DA column (60 m × 0.32 mm × 0.5 mm), a flame ionization detector, and nitrogen at 260°C as carrier gas; i-butanol was used as an internal standard. Chromatograms were analyzed using GC-Solution software (Shimadzu Corporation). Monosugars and lactate concentration were quantified with the Waters Alliance HPLC-System equipped with a Waters 2414 detector and a Reprosil-Pur NH2 column (250 mm × 4.0 mm × 5 µm) (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany), at 50°C with a flow rate of 1 mL/min for 20 min using acetonitrile:water:ethyl acetate (76:20:4).

RESULTS AND DISCUSSION

Bleaching

Figure 2 presents the development of the kappa number during bleaching, including the ISO brightness after the D1 stage. The PE-PSAQ pulps are somewhat easier to bleach. During oxygen delignification, the decrease in kappa number is about 7 units for the kraft pulp and about 9 units for the PE-PSAQ pulps. In the bleaching stages that follow, the kappa number decreases in a very similar trend, and brightness is higher for the PE-PSAQ pulps (77.7 and 76.2) than for the kraft pulp (71.5). **Table III** presents the residual chemicals and the pH values after the different bleaching stages. No residual chlorine was detected after the DO stage; after the other bleaching stages only small amounts of residual chemicals could be de-



2. Development of kappa number during bleaching and brightness after D1 stage.

Residual Chemicals and End pH	Kraft		PS0.5		PS2.0	
	Res Chem	pH	Res Chem	pH	Res Chem	pH
	g/L		g/L		g/L	
O2	-	10.4	-	10.5	-	10.4
O2-D0	0.00	2.7	0.00	2.4	0.00	2.4
O2-D0-Ep	0.07	10.0	0.11	9.9	0.07	9.7
O2-D0-Ep-D1	0.01	3.3	0.04	3.3	0.05	3.9
O2-D0-Ep-D1-P	0.10	12.3	0.08	12.5	0.02	12.6

III. Residual chemicals and pH after different bleaching stages.

termined, and all pH values were in the expected range.

In a study by Nevalainen and Hosia, the brightness of unbleached larch kraft pulp was lower than that of pine and spruce kraft pulp [27]. Hakkila et al., however, showed that the final brightness after bleaching (CEHDED) was almost equal for Siberian larch and Scots pine (*Pinus sylvestris*) [15]. This finding supports the claim that larch pulp is easier to bleach than pine or spruce pulp. While the brightness reversion was greater for larch pulps than for pine pulp, the yield loss was on the same level for both pulps [15].

Pulp and paper properties

Table IV presents the characteristics of the pulps and paper sheets from this study. The fiber properties were very similar for all three larch pulps. Somewhat lower SR values, which indicate better water removal, were seen for the PE-PSAQ pulps. The paper properties of the two larch PE-PSAQ laboratory sheets were generally very similar, while the larch kraft paper differed slightly from those of the pre-extracted pulps. The basic sheet properties were similar for all larch papers, although the PE-PSAQ sheets possibly had lower density. There was a clear difference in strength properties; the PE-PSAQ papers had lower tensile strength and higher tear strength than the larch kraft paper. The optical properties were all somewhat better for the PE-PSAQ papers; the PE-PSAQ pulps reached higher ISO brightness than the kraft pulp because of their better bleachability. In addition, the PE-PSAQ pulps had higher opacity and light scattering. The roughness and the air permeability were both higher for the

Pulp and Paper Properties			Kraft	PS0.5	PS2.0	Amount of Tests	StDev
Pulp & fiber	Kappa number		1.5	1.1	1.2	2	0.07
	Fiber length (L(l))	mm	2.55	2.55	2.48	3	0.03
	Coarseness	mg/m	0.202	0.202	0.212	3	0.00
	Fiber width	µm	29.3	29.3	29.0	3	0.15
	Zero span (dry) *)	Nm/g	144	144	153	10	8
	SR *)		18.9	18.9	15.3	1	-
General	Basis weight	g/m²	63.1	63.1	62.6	10 sheets pile weighed -> /10	
	Density	kg/m³	580	532	573	10 sheets pile measured -> /10	
	Bulk		6.9	7.4	7.0	10 sheets pile weighed -> /10	
Strength	Tensile	Nm/g	64.0	44.2	50.9	10	5.5
	Tear	mN	15.8	20.4	18.4	10	2.1
Opacity	ISO brigthness	%	77.4	81.6	80.7	0.06	0.06
	Opacity	%	62.7	66.2	65.1	10	1.58
	Light scattering	m_2/kg	20.5	24.6	22.8	Calculated based on brightness and opacity analyses.	
	Absorption	m_2/kg	0.18	0.14	0.15		
Surface	Roughness	mL/min	1945	2244	1844	10	169
	Air permeability	mL/min	1812	3383	2549	10	162

*) beaten pulp

IV. Pulp and paper properties of bleached laboratory larch pulps.

PE-PSAQ pulps, which can be explained by the lower hemicellulose content. Hemicelluloses were removed from the PE-PSAQ pulps during the pre-extraction, which led to a more porous paper.

Comparisons between the PS0.5 pulp and the PS2.0 pulp indicated the influence of the PS charge on the pulp and paper properties. The fiber properties were similar, except that the zero span seemed somewhat higher and the SR value was lower for the PS0.5 pulp (Table IV). When the paper properties of the PS0.5 pulp and the PS2.0 pulp were compared, the properties of the PS2.0 pulp were closer to the properties of the kraft pulp. The PS0.5 pulp was more porous than the PS2.0 pulp, which was a result of the difference in the PS charge. The PS charge in the PS2.0 pulp was higher; therefore, the glucomannans were more preserved than in the PS0.5 pulp.

Comparisons with other studies described the properties of the pulps and paper from this study in a broader perspective. A laboratory spruce kraft pulp [28] was beaten similarly as the larch pulps of our study (PFI, 2000 revolutions) and therefore was a good source for comparison. The dry zero span of the larch pulps was higher than the wet zero span of the spruce pulp (107 Nm/g), which indicated that the individual fibers in the larch pulps were stronger. Because only a minor difference should exist between the dry and wet zero span of undamaged laboratory pulps [29], a rough comparison between them could be made. The SR values were lower for the larch pulp than for the spruce pulp (22.4), especially for the PE-PSAQ pulps. The larch pulp fibers were somewhat longer than the spruce pulp fibers (2.29 mm) because less coarseness gives higher tensile strength [30]. The larch pulps were

Mass Balance	*L. brevis* ATCC367	*L. brevis* ATCC8287	*L. plantarum* DSM 20314	*B. coagulans* VKPM B-10468	*C. acetobutylicum* MSU 6	*C. Acetobutylicum* MSU 7	*C. acetobutylicum* VKPM B-4786	*C. saccharobutylicum* VKPM B-10183
IN fermentation								
Sugar, g/L	39.3	38.9	40.5	42.8	34.4	35.3	36.1	35.1
Arabinose	6.6	6.5	6.8	7.2	5.8	5.9	6.1	5.9
Galactose	26.1	25.7	26.9	28.4	22.8	23.4	24	23.3
Glucose	1.3	1.3	1.4	1.4	1.2	1.2	1.2	1.2
Xylose	2.0	2.0	2.0	2.2	1.7	1.8	1.8	1.8
Mannose	3.3	3.3	3.4	3.6	2.9	3.0	3.0	2.9
OUT fermentation								
Sugar, g/L	25.5	18.2	15.1	16.5	26.0	37.9	27.7	24.7
Arabinose	0.5	0.3	3.5	0.3	6.8	8.2	4.3	3.8
Galactose	20.5	14.6	8.7	13	16.5	24.3	22.2	19.9
Glucose	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0
Xylose	1.2	0.0	2.0	0.0	1.8	2.0	1.2	1.0
Mannose	3.3	3.3	0.7	2.9	0.0	2.3	0.0	0.0
Products, g/L	7.2	12.1	14.5	12.7	7.5	3.3	6.6	4.7
Lactate	2.9	4.8	9.5	4.8	4.7	0.2	0.2	0.1
Acetate	3.9	5.6	5.0	5.8	2.8	3.0	4.0	2.0
Ethanol	0.5	1.8	0.0	2.1	0.0	0.0	0.1	0.0
Butanol	0.0	0.0	0.0	0.0	0.0	0.0	0.1	1.0
Butyrate	0.0	0.0	0.0	0.0	0.0	0.1	2.2	1.6
Sugar utilized, g/L	13.7	20.6	25.6	26.6	nd	nd	8.4	10.4
Pentose utilized	6.9	8.2	3.3	9.1	nd	nd	2.4	2.9
Hexose utilized	6.8	12.4	22.3	17.5	10.4	nd	6.0	7.5
nd - Sugar concentrations cannot be determined because of hemicellulase activity of the strains.								

V. Results from fermentation of larch extract for 72 h of cultivation.

coarser than the spruce pulp (0.185 mg/m); therefore the spruce pulp had higher tensile strength (70.0 Nm/g) than the larch kraft pulp. The PE-PSAQ papers had significantly lower tensile strength, while their tear strength was higher than that of spruce kraft paper (17.6 mN). The poor tensile strength of the larch PE-PSAQ papers could be improved by further beating of the pulp. Beating affects tear strength and SR value of paper negatively; however, these properties were both better for the PE-PSAQ pulps and papers so further beating would be possible. Further beating would probably make the strength of the PE-PSAQ papers as good as that of the larch kraft or the spruce kraft paper. Spruce paper (719 kg/m^3) had higher density than the larch papers, as well as higher ISO brightness and opacity (88.5% and 70.0%, respectively).

In the study by Nevalainen and Hosia, larch unbleached kraft pulps were shown to have lower sheet density than Siberian larch, Scots pine, and Norway spruce pulps [27]. The lower sheet density of larch unbleached pulp compared to Norway spruce was also observed by Hatton et al. [31], probably due to higher fiber coarseness of larch pulps. In the study by Hakkila, larch pulps had somewhat better strength properties, especially tensile strength, than the larch pulps of our study (tensile 66–75 Nm/g and tear 19–20 nNm^2/g at brightness 66%–86% and SR 20) [15]. The bleaching sequences in Hakkila's experiments were different from those used in our study, which can partly explain the differences. For unbleached pulps the tensile strength was lower than that of the pine and spruce pulps beaten to the same level, while the tear strength was significantly higher [27]. The good tear strength of larch is due to its long fibers and thick-walled latewood fibers. Increasing tree age affects the strength properties by decreasing tensile strength and by increasing tear strength [15]. Industrial elemental chlorine free and totally chlorine free bleached pulp of *P. sylvestris* with tensile strength of 50–70 Nm/g had tear strength of 15–20 nNm^2/g [32]. The strength properties of the larch pulps in our study were within the same range as those of the industrial pine kraft pulps.

The results of our study agree with what is generally known about the influences of the amount of hemicelluloses on paper properties and characteristics [33]. Fewer hemicelluloses lead to less fiber swelling and lower interfiber bonding—the paper will have lower density, higher porosity, lower tensile strength, higher tear strength, and better optical properties. Because its fibers are long and strong, larch would be especially suitable for paper requiring good strength properties (e.g., packaging papers and boards as well as reinforcement pulp). Although there were small variances in the strength properties between the PE-PSAQ pulps and other softwood pulps, in general the PE-PSAQ pulps could be used for similar applications as other softwoods (i.e., pine or spruce).

Fermentation products

Table V presents the concentrations before and after fermentation. The sugar concentration of the initial extract was 35–43 g/L, mainly arabinogalactan. After fermentation the sugar concentration was 15–38 g/L, while the product concentration was 3–15 g/L. The main fermentation products were lactic acid and acetic acid. Generally, the hexoses were more actively used and galactose was rather inefficiently consumed by all the bacteria strains.

Of the *Lactobacillus* strains, *L. plantarum* DSM 20314 was the most efficient for fermenting the larch extract. It actively used hexoses (glucose, galactose, mannose), fermented arabinose less efficiently, and was not able to metabolize xylose. The two *L. brevis* strains and *B. coagulans* VKPM B-10468 revealed similar fermentation profiles, producing lactate, acetate, and ethanol. Arabinose was almost completely used in 72-h fermentation by all three strains. Mannose was not consumed at all by *L. brevis* strains. Rates of galactose usage were highest for *B. coagulans* VKPM B-10468 and lowest for *L. brevis* 5563 strains. Of note, the end-products profile of heterofermentative *B. coagulans* VKPM B-10468 strain differed from *B. coagulans* MXL-9 [5], producing mainly lactate.

All of the *Clostridium* strains showed weaker fermentation results. The sole product of *C. acetobutylicum* MSU 6 was lactate, and *C. acetobutylicum* MSU 7 weakly fermented sugars of hydrolyzed larch extract and produced acetate and butyrate in trace amounts. The slight increase in pentose concentration in the fermentation media of *C. acetobutylicum* MSU 6 and *C. acetobutylicum* MSU 7 at the end of cultivation may be explained by hemicellulase activity of the strains [25] in the presence of residual amounts of nonhydrolyzed oligosaccharides in the media. *C. acetobutylicum* VKPM B-4786 produced lactate, acetate, ethanol, butanol, and butyrate. *C. saccharobutylicum* VKPM B-10183 produced butyrate and butanol. *Clostridium* strains preferred glucose and mannose over galactose and arabinose. All of the *Clostridium* strains grew poorly on larch hydrolysate, probably because of the effect of inhibitors emerging from extraction and hydrolysis processes. However, the main inhibitors were not characterized in this study.

Table VI presents fermentation yields. The following yields were calculated: % on used sugars, % on initial sugars, and % on wood. Analyzing the yield as % on used sugars showed that acetate production by *C. acetobutylicum* VKPM B-4786 gave the highest percentage (47.7%), followed by the lactate production by *L. plantarum* DSM 20314 (37.2%). The latter case also showed the most attractive result as % on initial sugar (23.6%) and % on wood (1.7%).

Lactate was produced by *L. plantarum* DSM 20314, *L. brevis* ATCC367, *L. brevis* ATCC8287, *B. coagulans* VKPM B-10468, and *C. acetobutylicum* MSU 6. The highest fermentation yield for 72-h cultivation was obtained by producing lactate with *L. plantarum* (36.3% from used sugars) and *C. acetobutylicum* MSU 6 (35.6% from used sugars). Acetate was produced by all strains tested in this study. Ethanol was produced by two *L. brevis* strains, *B. coagulans* VKPM B-10468, and *C. acetobutylicum* VKPM B-4786. Butanol and butyrate

Fermentation yield	*L. brevis* ATCC367	*L. brevis* ATCC8287	*L. plantarum* DSM 20314	*B. coagulans* VKPM B-10468	*C. acetobutylicum* MSU 6	*C. Acetobutylicum* MSU 7	*C. acetobutylicum* VKPM B-4786	*C. saccharobutylicum* VKPM B-10183
% on utilized sugar	**52.8**	**58.9**	**56.6**	**47.6**	**nd**	**nd**	**78.4**	**45.5**
Lactate	20.9	23.1	37.2	17.9	nd	nd	2.3	1.0
Acetate	28.4	27.2	19.4	21.8	nd	nd	47.7	19.4
Ethanol	3.5	8.6	0.0	7.9	nd	nd	0.6	0.4
Butanol	0.0	0.0	0.0	0.0	nd	nd	1.4	9.4
Butyrate	0.0	0.0	0.0	0.0	nd	nd	26.4	15.3
% on initial sugar	**18.4**	**31.2**	**35.8**	**29.6**	**21.7**	**9.4**	**18.3**	**13.5**
Lactate	7.3	12.2	23.6	11.1	13.6	0.6	0.5	0.3
Acetate	9.9	14.4	12.3	13.6	8.0	8.5	11.1	5.8
Ethanol	1.2	4.6	0.0	4.9	0.0	0.0	0.1	0.1
Butanol	0.0	0.0	0.0	0.0	0.0	0.1	0.3	2.8
Butyrate	0.0	0.0	0.0	0.0	0.1	0.2	6.2	4.5
% on wood	**1.4**	**2.3**	**2.7**	**2.2**	**1.6**	**0.7**	**1.4**	**1.0**
Lactate	0.5	0.9	1.7	0.8	1.0	0.0	0.0	0.0
Acetate	0.7	1.1	0.9	1.0	0.6	0.6	0.8	0.4
Ethanol	0.1	0.3	0.0	0.4	0.0	0.0	0.0	0.0
Butanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Butyrate	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3

nd - Sugar concentrations cannot be determined because of hemicellulase activity of the strains.
Extract contains 7.4% on wood sugar -> Ferm. yield: % on wood = 7.4 * % on initial sugar.

VI. Fermentation yields of larch extract for 72 h of cultivation.

were produced by *C. acetobutylicum* VKPM B-4786 and *C. saccharobutylicum* VKPM B-10183 strains.

Earlier fermentation experiments by our research group gave more attractive results [5], where a larch extract containing 36 g/L sugars was fermented into 28 g/L lactic acid with *B. coagulans* MXL-9. The corresponding yields for 72-h cultivation time for the *B. coagulans* MXL-9 case were 64.4% on initial sugar and 4.8% on wood and 97 h 78.5% on initial sugar and 5.8% on wood, respectively. It appeared that *B. coagulans* MXL-9 more efficiently converted both C5 and C6 sugars into lactic acid compared to all the different bacteria strains tested in this study. To increase the fermentation efficiency, the larch medium should be supplemented with appropriate sugar and media components. In addition, detoxification to remove the inhibitors may be necessary.

CONCLUSIONS

Comparisons between PE-PSAQ paper and kraft paper of larch wood showed that the PE-PSAQ process did not affect the paper properties negatively. Instead, most properties were similar or even better than the properties of the larch kraft paper. The properties of the PE-PSAQ paper were also compared to paper properties of other softwood species. On the basis of our study results, larch PE-PSAQ pulp could be used for paper products similar to those from spruce or pine kraft pulps, such as for packaging boards and papers and also as reinforcement pulp. The following is a summary of bleaching results and paper properties:

1. PE-PSAQ larch pulps are easier to bleach than kraft larch pulps.
2. The SR value is lower for the PE-PSAQ pulps than for the kraft pulp, which means better water removal for the pre-extracted pulps.
3. PE-PSAQ pulps have somewhat lower sheet density than the kraft pulp.
4. PE-PSAQ pulps have poorer tensile strength but better tear strength than larch kraft pulps.

5. PE-PSAQ pulps reached higher brightness than the kraft pulps, because the PE-PSAQ pulps are easier to bleach.
6. Opacity, the light scattering, and air permeability were higher for the PE-PSAQ pulps.

Eight different bacteria strains were tested to produce different products from the larch extract through fermentation. In general, *Lactobacillus* strains metabolized sugars of larch extract faster than *Clostridium* strains. The tested strains had different sugar specificity: *L. plantarum* DSM 20314 fermented all HLE sugars except xylose; *L. brevis* strains did not use mannose; *B. coagulans* and the *Clostridium* strains (except for *C. acetobutylicum* MSU 7) metabolized all HLE sugars; and *C. acetobutylicum* MSU 7 fermented larch extract very weakly. Lactate production by *L. plantarum* DSM 20314 yielded the most attractive result in this study.

There is potential for the use of larch wood with the PE-PSAQ process, where paper would remain the main big-volume product and lactic acid would be produced by *B. coagulans* MXL-9 fermentation as a value-added product from the side sugar streams. **TJ**

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ABOUT THE AUTHORS

Our specific interest in this research topic lies in the large amount of water-soluble compounds of larch wood, which could be used to advantage in a forest biorefinery producing biochemicals. The focus was on the products of a larch biorefinery because other parts of this biorefinery process were analyzed and optimized earlier by this research team. Research on larch has been done in Russia, which has large resources of the wood. Globally, not many studies have been done on larch wood.

The most challenging aspect of this research was to provide enough evidence of the process so that partners from the industry would be willing to apply it. We tried to develop the process using practical conditions and charges. We determined the proof of concept for this process. We consider the possibility of maintaining pulp yield, despite removing most of the soluble hemicelluloses from the wood before the pulping process, to be very interesting.

Pulp mills with access to larch resources might consider adding a prehydrolysis stage to their cooking process to produce lactic acid as a value-added product in addition to paper-grade pulp. Next, this larch biorefinery process will undergo economic evaluation.

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