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Biorefining of wheat straw using an acetic and formic acid based organosolv fractionation process

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HIGHLIGHTS

- Wheat straw was fractionated by an acetic and formic acid based organosolv process.
- A thorough mass balance of main compounds in the different fractions was made.
- Cellulose and lignin were efficiently recovered in different process fractions.
- The process led to extensive hydrolysis of hemicellulose.
- The organosolv process is a solid base for biorefining.

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ABSTRACT

To assess the potential of acetic and formic acid organosolv fractionation of wheat straw as basis of an integral biorefinery concept, detailed knowledge on yield, composition and purity of the obtained streams is needed. Therefore, the process was performed, all fractions extensively characterized and the mass balance studied. Cellulose pulp yield was 48% of straw dry matter, while it was 21% and 27% for the lignin and hemicellulose-rich fractions. Composition analysis showed that 67% of wheat straw xylan and 96% of lignin were solubilized during the process, resulting in cellulose pulp of 63% purity, containing 93% of wheat straw cellulose. The isolated lignin fraction contained 84% of initial lignin and had a purity of 78%. A good part of wheat straw xylan (58%) ended up in the hemicellulose-rich fraction, half of it as monomeric xylose, together with proteins (44%), minerals (69%) and noticeable amounts of acids used during processing.

1. Introduction

Wheat (*Triticum aestivum* L.) straw is the most abundant biomass feedstock among agricultural residues in Europe (Kim and Dale, 2004) and therefore considered to be interesting for the production of second generation bioethanol (Talebnia et al., 2010) or for biorefining in general (Cherubini and Ulgiati, 2010). Wheat straw mainly consists of cellulose (28–39%), hemicellulose (23–24%) and lignin (16–25%), but considerable levels of ash (6.4–9.7%) and protein (4–5%) are also present (Carvalheiro et al., 2009). To efficiently use wheat straw for biorefinery, it needs to be fractionated into its major constituents, i.e. cellulose, hemicellulose and lignin. This pretreatment or fractionation process plays a vital role in biorefinery as the purity of the obtained fractions will impact their value for further valorization (Huijgen et al., 2012). In the case of subsequent enzymatic cellulose hydrolysis, the pretreatment of lignocellulosic biomass is crucial to make the crystalline microfibrils, which are trapped in a hemicellulose-lignin matrix, enzyme digestible. To make such a biorefinery process economically feasible, the pretreatment of lignocellulosic biomass should provide highly digestible sugar fractions and lignin that can be converted to valuable co-products. Degradation of sugars and formation of inhibitors should be avoided or kept to minimum

Abbreviations: AIL, acid insoluble lignin; ASL, acid soluble lignin; CIMV, Compagnie Industrielle de la Matière Végétale; ICP-AES, inductively coupled plasma atomic emission spectroscopy.

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as this impairs further conversion (Brodeur et al., 2011; Mosier et al., 2005).

Organosolv fractionation is a promising fractionation technology and comprises a class of processes in which lignocellulosic biomass is treated with a mix of an organic solvent and water, often at elevated temperatures (Kumar et al., 2009; Xu et al., 2006). Commonly used solvents are ethanol, methanol, acetone and organic acids like acetic acid and formic acid or combinations thereof (Xu et al., 2006). Due to the process, cellulose is delignified, with the organic solvent functioning as lignin extraction solvent, and the hemicellulose is depolymerized through acid hydrolysis by the added acid or the acid that is formed from the acetyl side groups of the hemicellulose at elevated temperature (Huijgen et al., 2011). In general, organosolv processing aims to fractionate the lignocellulosic biomass as much as possible into its individual major fractions. This stands in sharp contrast with currently practiced fractionation technologies such as steam explosion, acid and enzymatic hydrolysis, that merely liberate the cellulose fraction for further processing without specific recovery of hemicellulose and lignin. Consequently, the application spectrum for the more pure organosolv fractions is broader when compared to the impure fractions derived from conventional pretreatments, such as acid catalyzed steam explosion, enzymatic hydrolysis and fermentation which are targeted towards the production of ethanol. The residual fraction is a complex mixture of unconverted carbohydrates, lignin and process chemicals. To date no value-added applications for such complex by-products have been identified, except CHP to generate heat.

Some carboxylic acid based processes, such as Acetosolv, Formacell and Milox, have already been put forward as promising pulping processes due to their purification selectivity and lower specific investment costs (Sixta et al., 2004). The European Union FP7 project "BioCOmmodity REfinery" (or in short "BIOCORE") (2010–2014) aims to create and demonstrate a lignocellulosic biorefinery for sustainable processing using an innovative acetic and formic acid based organosolv fractionation process developed by Compagnie Industrielle de la Matière Végétale (CIMV) on pilot scale (Benjelloun-Mlayah and Delmas, 2010; Benjelloun-Mlayah et al., 2005). Due to the use of a mixture of acetic acid and formic acid in the CIMV process, the biomass refining can be performed at atmospheric pressure and a temperature lower than 110 °C, avoiding degradation of the hemicellulose sugars (especially xylose). The process had been initially developed for producing paper pulp from wheat straw and was optimized within the BIOCORE project for several other agricultural residues (e.g. rice straws), short rotation coppice (e.g. poplar) and hardwood forestry residues.

The cellulose pulp, lignin and hemicellulose fractions obtained in this process will be used further for the production of a variety of chemicals (including polymers and specialty molecules), materials (including biodegradable packaging or insulation material) and energy (including second generation biofuels, heat and power), but falls out of the scope of this manuscript.

To asses the potential of the acetic and formic acid organosolv fractionation of wheat straw as basis of an integral biorefinery concept, more in-depth knowledge on yield, composition and purity of the different streams obtained in this process is needed. The aim of this manuscript is therefore to get more insight in the distribution of wheat straw components over different organosolv fractions using the above mentioned organosolv process and to make an extensive mass balance of wheat straw fractionation, using a lab scale setup. Characterization of the different fractions will provide information on the performance of the process. Knowledge of the composition of the produced fractions will in part determine their further application potential.

2. Methods

2.1. Materials

All solvents, chemicals and reagents were of at least analytical grade. Wheat straw was received ambient-dry from the Champagne-Ardennes region, France (harvested in summer 2009). Before usage in the organosolv process, wheat straw was mechanically cut in wisps of 5–15 cm length, using a hammer crusher, and the fines (<4 mm) were removed by cycloning. Before analysis, straw was further milled to powder using a cutting mill.

2.2. Acetic and formic acid based organosolv process

A process scheme of the CIMV acetic and formic acid organosolv process is depicted in Fig. 1. In a first step, lignin and hemicellulose fractions were solubilized by impregnating wheat straw (289 g dry matter) with a mix of acetic acid and formic acid (65/35 mass ratio, 85% in water) in a total volume of 3.0 L at 105 °C for 3 h.

The mixture was membrane filtered and the residual raw pulp further delignified by the action of peracetic and performic acid, *in situ* generated after hydrogen peroxide addition (10% on dry matter raw pulp, 85 °C, 90 min), to obtain the cellulose pulp. Finally, the pulp was washed twice with the aqueous acid organosolv solution (see above) and water until a pH between 6.5 and 7.0 was reached, respectively. After membrane filtration, the washing fluid containing acids was combined with the hemicellulose and lignin extraction liquor, while the washing water was collected in the waste water.

Acids were evaporated from the hemicellulose and lignin containing extraction liquor by vacuum evaporation (60 °C, 320 mbar), until a dry matter content of 60% was reached. Evaporated water was collected as waste water while the organic acids were recovered and could be reused in a consecutive process. Lignin was coagulated as small particles in the media by adding water (water/concentrated extraction liquor ratio of 1/1) while mechanically stirring and were separated from the supernatant by membrane filtration at 45 °C. The lignin fraction was washed with water and pressed to concentrate the lignin fraction and remove the process water. The diluted hemicellulose-rich fraction (supernatant) was further concentrated up to 55% dry matter content by subsequent vacuum evaporation (50 °C, 320 mbar) and deacidification by means of steam stripping (steam to feed ratio 1.5, 70 °C).

2.2. Analytical methods

2.3.1. Carbohydrate content and composition

For analysis of total carbohydrate content and composition, including cellulose, total hydrolysis conditions were used (Gourson et al., 1999). Samples (5–20 mg) were pre-hydrolyzed for 2 h in 13.0 M sulfuric acid (1.0 mL) at room temperature, then hydrolyzed in 2.0 M sulfuric acid (6.5 mL) at 100 °C for 2 h. Following hydrolysis, resulting monosaccharides were reduced to alditols and acetylated as described by Courtin et al. (2000). To this end, internal standard (1.0 mL, 1.0 mg/mL β-D-allose in 50% benzoic acid solution) was added to 3.0 mL of hydrolyzed sample. Concentrated NH_3 (1.0 mL) and droplets of octanol were added before reduction with sodium borohydride (0.2 mL, 200 mg in 1.0 mL NH_3 2.0 M) for 30 min at 40 °C. The reaction was stopped by adding 400 μL acetic acid. A catalyst, 1-methylimidazole (500 μL), was added to the reduced samples (500 μL) to facilitate the formation of alditol acetates after addition of acetic acid anhydride (5.0 mL). After 10 min, 900 μL of ethanol was added and reaction was stopped by adding 10.0 mL water 5 min later. Afterwards bromophenol blue (0.5 mL;

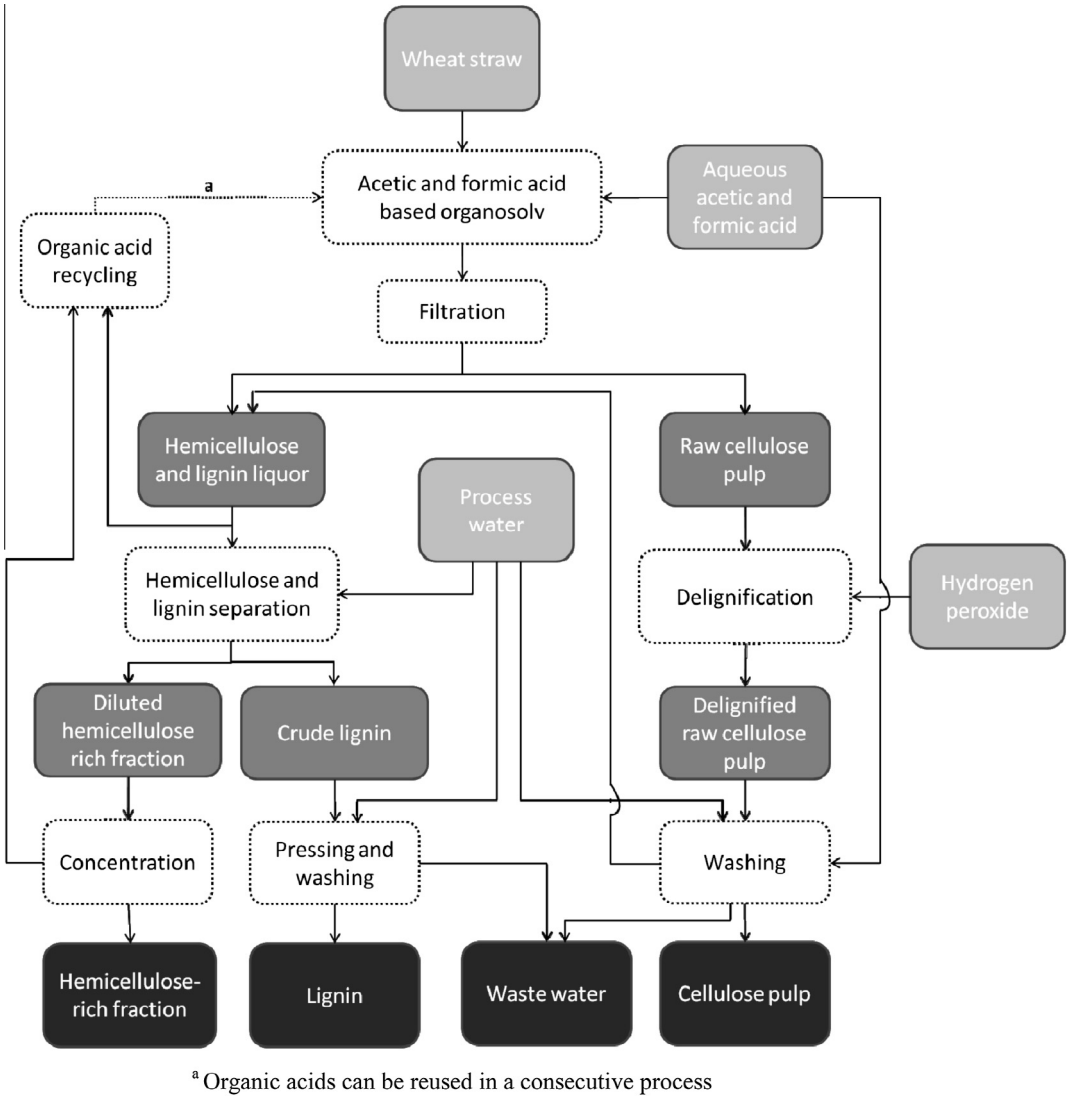

Fig. 1. Overview of the acetic and formic acid based organosolv fractionation of wheat straw and its derived fractions.

0.04 g in 100 mL water) and potassium hydroxide (two times 5.0 mL, 7.5 M) were added to color the aqueous phase and hence facilitate the removal of the organic ethylacetate phase by a pasteur pipette. The organic phase was dried with anhydrous Na_2SO_4 and put into a vial. Gas chromatographic analysis of fully acetylated sugar alcohols (1.0 μL) was performed on a Supelco SP-2380 column (30 m × 0.32 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, PA, USA) with helium as carrier gas in a Agilent 6890 series chromatograph (Agilent, Wilmington, DE, USA) equipped with an autosampler, splitter injection port (split ratio 1:20), and flame ionization detector. Separation was at 225 °C with injection and detection temperatures at 270 °C. Calibration samples, containing known concentrations of the expected monosaccharides and the internal standard, were included with each set of samples. For determination of free monosaccharide content, no hydrolysis took place. Upon total carbohydrate content determination, corrections for the presence of the monosaccharide building blocks as anhydrosugars in polymeric carbohydrates were made.

2.3.2. Lignin content

Determination of the acid-insoluble (AIL) and acid-soluble lignin (ASL) content was performed in duplicate as described by Huijgen et al. (2012). After removal of the extractives by H_2O and ethanol extractions, samples (300 mg) were hydrolyzed in two steps: (1) pre-hydrolysis using 3.0 mL 12.0 M (72% w/w) sulfuric acid (30 °C, 1 h) and (2) final hydrolysis after tenfold dilution with water (final volume 30.0 mL, 1.2 M sulfuric acid, 100 °C, 3 h). The solid residue was determined gravimetrically and its ash content measured by combustion at 550 °C according to protocol NREL/TP-510-42622 (NREL (National Renewable Energy Laboratory, 2009)). The AIL content corresponds to the amount of ash-free residue (not corrected for its protein content). The hydrolyzate was analyzed spectrophotometrically for its ASL content [UV–VIS absorption at 205 nm, absorption coefficient of 110 L g^{-1} cm^{-1} used according to TAPPI UM-250 (Technical Association of the Pulp and Paper Industry (TAPPI, 1976))]. Additionally the waste water was measured for its ASL content by the same method.

The lignin content in the cellulose pulp fraction was determined by the kappa number divided by 6. To determine this kappa number, about 3 g of wet pulp was treated with potassium permanganate ($KMnO_4$) in accordance with TAPPI Classical Method T 236 cm-85 "Kappa Number of Pulp".

2.3.3. Protein content

Protein contents were determined according to a Dumas combustion method, an adaptation of the Association of Official

Analytical Chemists method for protein determination (AOAC, 1995) using an automated Dumas protein analysis system (EAS varioMax N/CN, Elt), and using 6.25 as the nitrogen protein conversion factor.

2.3.4. Organic acid content

Total acetic and formic acid contents of samples (50 mg) were determined after saponification in 0.8 M sodium hydroxide (1.0 mL), on ice for 1 h and at room temperature for 2 h (Van Gool et al., 2011). Hydrogen chloride (200 μL, 4.0 M) was added to acidify samples to a pH below 7. Succinic acid (100 μL, 5.0 mg/mL) was added as internal standard and samples were filtered (0.45 μm) before analysis. Acids were separated as described by Jayaram et al. (2013) using a LC-20AT modular HPLC system (Shimadzu, Kyoto, Japan) using an ion exclusion ROA-Organic acids column (Phenomenex) and detected with refractive index detection (Shimadzu, RID-10A detector) using the following conditions: 60 °C column temperature, aqueous 2.50 mM sulfuric acid as eluent and a flow rate of 0.60 mL/min. For determination of free acetic acid, 50 mg sample was dissolved in 1.0 mL water and succinic acid (100 μL, 5.0 mg/mL) was added before analysis. Calibration samples containing succinic, acetic and formic acid were included with each set of samples.

Total ferulic and coumaric acid content was determined starting by saponification of 10 mg sample in 2.0 M sodium hydroxide (2.0 mL) under slow stirring for 2 h at 35 °C under N_2-atmosphere, protected from light (Antoine et al., 2003). After acidification to pH 2.0 with 4.0 M hydrogen chloride (approximately 1.05 mL), caffeic acid (100 μL, 0.5 mg/mL methanol) was added as internal standard. Phenolic acids were extracted twice with diethyl ether (2.0 mL) and ether was subsequently evaporated under a nitrogen flow. Phenolic acids were dissolved in methanol, filtered (0.45 μm) and analyzed on a Luna Phenyl-Hexyl column (250 × 4.6 mm i.d., 5 μm particle size, plus 3 × 4.6 mm i.d. guard column; Phenomenex, Utrecht, The Netherlands) as described by Dobberstein and Bunzel (2010) using a ternary gradient system [eluent (A) 1 mM aqueous trifluoroacetic acid; eluent (B) acetonitrile/1 mM aqueous trifluoroacetic acid (90/10 v/v); eluent (C) methanol/1 mM aqueous trifluoroacetic acid (90/10 v/v)] at a flow rate of 1.0 mL/min. Injection volume was 20 μL and the separation was performed at 45 °C. Chromatograms were recorded at 280 nm. Calibration samples containing all expected phenolic acids and caffeic acid were run with each set of samples.

The uronic acid contents in samples was determined using the *m*-hydroxydiphenyl method, as described by Blumenkrantz and Asboe-Hansen (1973) with some modifications, using glucuronic acid as standard. Samples (50 mg) were subjected to the same hydrolysis conditions as mentioned above for gas chromatographic analysis. After hydrolysis, deionized water was added up to a total volume of 50 mL after filtering. Hydrolyzate (600 μL) was transferred to a glass test tube and placed in an ice bath to cool. After addition of 3.6 mL sulfuric acid/tetraborate solution (12.5 mmol L^{-1} sodium tetraborate in 96% sulfuric acid), the mixtures were shaken and placed in a water bath at 100 °C for 6 min. After cooling and filtration, *m*-hydroxydiphenyl reagent (60 μL, 0.15% meta-hydroxydiphenyl in 0.5% NaOH) was added. The samples were mixed and extinction measurements were done one minute after addition of the reagent at 520 nm (Ultrospec III UV/vis spectrophotometer, GE Healthcare, Piscataway, USA).

2.3.5. Mineral content

Total mineral content was determined by thermogravimetric analysis (PerkinElmer TGA7) upon heating of about 10 mg of dried sample at a rate of 10 °C per minute from room temperature till 900 °C in an air/nitrogen atmosphere till constant weight.

The inorganic elemental composition was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The waste water sample was boiled in *aqua regia* (HNO_3/HCl in 1/3 volume ratio) before ICP analysis (Varian Vista AX). The hemicellulose-rich fraction was measured directly, while the remaining samples were dried, finely milled and subsequently digested using HNO_3/$HClO_4$/HF before ICP analysis (Thermo ICAP 6000). In addition, Cl was determined using ion chromatography according to NEN-EN-ISO 10304-1 (Dionex IC25, column Dionex AS18) [for the solid samples following bomb combustion in a calorimeter (Parr 6300) and subsequent water washing of the combustion residues].

3. Results and discussion

The objective of the acetic and formic acid based organosolv fractionation process is to separate the major components of wheat straw, i.e. cellulose, lignin and hemicellulose, for further processing to chemicals, materials and biofuels. After performing the procedure, about 48% of the wheat straw dry matter ended up in the cellulose pulp, 21% in the lignin fraction and 27% in the hemicellulose-rich fraction (Table 1). Losses of dry matter in the process were minimal as only 4% of initial dry matter was recovered in waste water (Table 1). Composition of these fractions and distribution of wheat straw components over different fractions are discussed below. Further adjustments on the process on pilot-scale and possibilities for further processing of the produced fractions are discussed as well.

3.1. Composition of wheat straw and the organosolv process derived streams

The lab-scale acetic and formic acid based organosolv fractionation process on wheat straw yielded 4 different fractions, i.e. cellulose pulp, a lignin cake, a hemicellulose-rich syrup and waste water (Fig. 1). Tables 2 and 3 summarize the composition of these organosolv process derived fractions in comparison to that of the starting material, wheat straw.

The major component of wheat straw was polymeric glucose (34%, determined as the amount of glucose after hydrolysis and corrected for incorporation of water during hydrolysis), mainly present as cellulose. Hemicellulose (24%, determined as the sum of arabinose and xylose content after hydrolysis and corrected for incorporation of water during hydrolysis) and lignin (18%, sum of AIL and ASL) were the second and third most abundant components. Minerals (4%), proteins (4%) and organic acids [4%, sum of acetic (mainly present as acetyl groups), formic, uronic, ferulic and coumaric acid] were present in lower amounts. These results were in good agreement with the composition of the same wheat straw as published by Wildschut et al. (2013). The sum of identified components present in the wheat straw was 89%. Potassium (6.2 g/kg dry matter), silicon (4.7 g/kg dry matter) and calcium (4.5 g/kg dry matter) were the most abundant elements in wheat straw.

The cellulose pulp had a purity of 63% (polymeric glucose content corrected for the incorporation of water upon hydrolysis). The polymeric xylose content amounted to 13%. About 1.6% bound acetic acid was present in the cellulose pulp, most likely associated with the xylan as acetyl group. The lignin content, determined as the kappa number divided by 6, amounted to 1.7%. Finally, low amounts of ash [1.5%, of which silicon (6.5 g/kg dry matter) was enriched compared to wheat straw and was the far most abundant element in this fraction], uronic acids (1.5%) and formic acid (1.4%) were also present. Only traces of free sugars were found in the cellulose pulp. The sum of identified components present in the cellulose pulp was 87%.

Table 1
Proportional distribution (% of component in wheat straw) of the major components of wheat straw in the different fractions resulting from the acetic and formic acid based organosolv process based on the recovered amounts.

(%)	Cellulose pulp	Lignin fraction	Hemicellulose-rich fraction	Waste water
Total dry matter	48	21	27	4
Total glucose	93	1	5	1
Total xylose	33	2	58	7
Lignin[a,b]	4	84	12	–
Protein[b]	0	56	44	–
Minerals[b]	23	8	69	–

[a] Lignin content as sum of acid soluble and acid insoluble lignin or the kappa number divided by 6.
[b] Waste water was not taken into account as compound was not quantified in this fraction.

Table 2
Composition (% w/w dry matter) of wheat straw and acetic and formic acid organosolv process derived fractions.

	Wheat straw (% w/w dm)	Cellulose pulp (% w/w dm)	Lignin fraction (% w/w dm)	Hemicellulose-rich fraction (% w/w dm)	Waste water (% w/w dm)
Total carbohydrates[a]	59.9	77.6	5.9	58.3	58.0
Total/free glucose	37.7/0.1	70.3/0.0	2.6/0.0	6.6/0.9	5.6/0.9
Total/free xylose	23.8/0.0	15.3/0.0	2.3/0.0	47.0/24.5	42.8/26.0
Total/free arabinose	3.7/0.1	0.3/0.0	1.3/0.5	8.5/6.5	8.5/7.1
Total/free galactose	1.2/0.0	0.1/0.0	0.1/0.0	3.4/1.4	2.8/1.4
Total/free mannose	0.6/0.2	0.6/0.0	0.1/0.0	1.1/0.7	1.5/0.8
Total/free acetic acid	0.8/0.3	3.8/2.2	0.7/0.1	10.1/0.8	–
Formic acid	0.0	1.4	0.0	8.9	–
Uronic acid	2.7	1.5	0.4	5.9	4.1
Coumaric acid	0.29	0.01	0.43	0.32	0.23
Ferulic acid	0.23	0.01	0.22	1.12	1.36
ASL[b]	1.2	1.7 [d]	1.4	4.7	0.2
AIL[c]	16.5		77.1	3.5	–
Proteins	3.8	0.0	8.0	4.8	–
Minerals	3.5	1.5	1.1	8.1	–
Total	89	87	101	110	67

[a] Corrected for their presence as anhydrosugars in polymers.
[b] ASL, acid soluble lignin.
[c] AIL, acid insoluble lignin.
[d] Total lignin content determined by the kappa number divided by 6.

Table 3
Inorganic elemental composition (mg/kg dry biomass) of wheat straw, acetic and formic acid organosolv process derived fractions.

	Wheat straw (mg/kg dm)	Cellulose pulp (mg/kg dm)	Lignin fraction (mg/kg dm)	Hemicellulose-rich fraction (mg/kg dm)	Waste water (mg/kg)[a]
Ca	4465	615	1265	17038	52
Cl	499	185	335	3209	11
Fe	71	106	1418	3739	7
K	6241	32	40	29755	42
Mg	698	41	81	2820	7
Na	53	40	37	1936	11
S	642	68	1809	2102	8
Si	4729	6468	712	235	3

[a] Due to the high dilution rate, elemental composition was not expressed on dry matter base.

The lignin fraction had a purity of 78% (sum of AIL and ASL) and also contained proteins (8%), which might have co-condensated with lignin (further discussed below), carbohydrates (5.3%, mainly oligomers of xylose, glucose and/or arabinose) and minerals (1.1%). Acetic, uronic acid and phenolic acid groups were only present in low concentrations (approximately 0.5%). The sulfur content only amounted to 0.2%, in contrast to higher sulfur contents in Kraft process derived thiolignin (Vishtal and Kraslawski, 2011). The sum of identified components present in the lignin fraction was 101%.

The main constituent of the hemicellulose-rich fraction was xylose (47%), but other sugars like arabinose (8.5%), glucose (6.6%), galactose (3.4%) and mannose (1.1%) were also present. Due to the acidic conditions applied during fractionation, hemicellulose derived sugars were present in monomeric and oligomeric form. More than half of the xylose and arabinose residues were present as monomeric sugars while glucose was mainly present as oligomers. Furthermore, these carbohydrates were more highly substituted with acetic (9.3%) and uronic acid (5.9%) compared to those in native wheat straw, indicating that the organosolv process conditions led to acetylation of sugar moieties. While both acetic and formic acid were used in the organosolv fractionation process, the detected acetate was mostly bound to the sugar fraction during the process (only 0.8% of free acetate remained present) making formic acid the most abundant carboxylic acid present in hemicellulose-rich fraction (8.9%). Also phenolic acids like coumaric (0.32%) and ferulic acid (1.12%) were present in the hemicellulose-rich fraction. Furthermore, relatively high amounts of ash [8.1%, with potassium

(29.8 g/kg dry matter) and calcium (17.0 g/kg dry matter) as most abundant elements], lignin (8.2%) and proteins (4.8%) were found in this fraction. Overestimation of total dry matter content (110%) could have resulted from not correcting for bound constituents, as acetic, uronic and phenolics acids were probably present as substituents on carbohydrates, and small analytical errors.

The composition of the waste water was rather similar to that of the hemicellulose-rich fraction, possibly because the hemicellulose-rich fraction contained more water soluble compounds than the lignin fraction and cellulose pulp. Some analyses could not be performed on the waste water because of its low dry matter content resulting in only 67% characterization of the waste water dry matter content.

3.2. Mass balance of the acetic and formic acid based organosolv fractionation process

During the first step of the organosolv fractionation process and subsequent delignification and washing of the raw pulp, 67% of hemicellulose (based on data of its main constituent xylose) and 96% of lignin were solubilized, whereas almost no cellulose was solubilized, resulting in cellulose pulp which contained 93% of initial glucose and 48% of wheat straw dry matter (Table 1). Also considerable amounts of initial hemicellulose derived carbohydrates (33%, based on the xylose distribution) and minerals (23%), and to a lesser extent lignin (4%), ended up in the cellulose pulp.

Wildschut et al. (2013) described a process optimization study for an (acid-catalyzed) ethanol-based organosolv fractionation process performed on the same wheat straw as used in this study. In comparison to their process optimized for subsequent enzymatic cellulose hydrolysis, the CIMV acetic and formic acid organosolv process in combination with the additional peracetic and performic acid delignification step showed more extensive delignification (96% vs. 76%). However, their process led to a higher degree of xylan removal (95% vs. 67%), leading to higher purity of the cellulose pulp compared to cellulose pulp produced in this study (75% vs. 63%).

About 21% of wheat straw dry matter was recovered in the lignin fraction, which contained most of the wheat straw lignin (84%). Also most of wheat straw proteins (56%), probably due to co-condensation to lignin, and minor portions of minerals (8%), xylan (2%) and glucan (1%) ended up this fraction.

About one fourth of the wheat straw mass was recovered in the hemicellulose-rich fraction. A high part of wheat straw hemicellulose (58%, mostly oligo- and monosaccharides) ended up in the hemicellulose-rich fraction, which is higher than the recovery of xylose in the hemicellulose-rich fraction (30%) obtained in the above mentioned ethanol organosolv process (Wildschut et al., 2013). Also large portions of wheat straw minerals (69%) and proteins (44%) and part of the original lignin (10%) and glucan (5%) ended up in this fraction.

Table 4 lists the total mass balance, based on the distribution of dry matter over the different fractions and the composition of wheat straw and these fractions. Initially, 289 g dry matter straw entered the process before acetic and formic acid was added, while a total of 285 g of dry matter was found back in the different fractions and waste water. It is plausible that carbohydrate-derived degradation products like furfural and hydroxymethylfurfural were formed, as a small fraction of sugars (7%) was not recovered. Indeed, furfural is a typical degradation product of acid based sugar hydrolysis and will typically be recovered in the aqueous hemicellulose-rich stream of organosolv processes (Kumar et al., 2009). The total amount of lignin determined in the different fractions was slightly higher than initially determined in wheat straw. Proteins can co-condensate with lignin under acidic conditions (Whitmore, 1982), such as occurring during the organosolv process. Condensation of lignin with degradation products of carbohydrates (e.g. furfural), acids and extractives is also possible. These condensation reactions probably resulted in the overestimation of the lignin content in the lignin determination and thereby a lignin fraction mass balance exceeding 100% (Wildschut et al., 2013). A small loss in proteins and minerals was noticed after fractionation. More acetic acid (both free as well as bound) and formic acid was found in the fractions compared to the wheat straw, due to the acids added during processing.

3.3. Further optimization of the process on pilot-scale and application assessment of derived fractions

This study, conducted at lab-scale, shows the efficiency of wheat straw refining using a mix of acetic acid and formic acid. During scale-up of its process, CIMV has been able to make some adaptations to the process that improved the efficiency of the refining. At pilot-scale, the extraction of hemicelluloses and lignin is conducted in a diffuser system, instead of in a reactor. Hereby, these two fractions are extracted in counter current mode, resulting in a cellulose fraction with a purity above 85% (vs. 63% in this study). To separate the lignin and hemicellulose fraction at pilot scale, a special mechanical disperser, instead of a mechanical stirrer at lab scale, is used. Lignin produced at pilot scale has a typical purity of above 85% (vs. 78% in this study). Purification of the hemicellulose-rich fraction can be done by ion exchange treatments.

Within the EU FP7 project BIOCORE, resulting fractions of the CIMV process are further studied for a wide variety of applications. Purity and composition of the cellulose pulp impact its further use. A possible application for the cellulose pulp is enzymatic hydrolysis of the polysaccharides to produce (fermentable) sugars. Typically, organosolv-derived cellulose is highly susceptible

Table 4
Overall mass balance of the lab scale acetic and formic acid organosolv fractionation of wheat straw. Amounts are expressed in grams. The loss is determined as the difference between the amount of the compound in wheat straw and the sum of the amounts present in the different fractions over the initial amount in wheat straw, expressed in percentage.

	Wheat straw (g)	Cellulose pulp (g)	Lignin fraction (g)	Hemicellulose-rich fraction (g)	Waste water (g)	Sum (g)	Loss (%)
Total dry matter	289	137	59	77	11	285	2
Total carbohydrates[a]	172.8	113.3	3.5	48.3	6.6	164.8	7
Total glucose	108.8	96.4	1.6	5.1	0.6	103.7	5
Total xylose	68.7	20.9	1.4	36.3	4.6	58.5	8
Total/free acetic acid	2.2/0.8	5.2/3.0	0.4/0.0	7.8/0.6	–	13.4/9.8	−631/−781
Formic acid	0.0	1.9	0	6.9	–	8.8	–
Lignin[b]	50.9	2.3	46.2	6.3	–	54.8	−8
Proteins	11.0	0.0	4.7	3.7	–	8.4	23
Minerals	10.1	2.1	0.7	6.3	–	9.0	11

[a] Corrected for their presence as anhydrosugars in polymers.
[b] Determined as sum of acid soluble and acid insoluble lignin or the kappa number divided by 6 (cellulose pulp).

to enzymatic hydrolysis (Chum et al., 1988). Several factors have been reported to influence enzymatic cellulose digestibility, such as cellulose crystallinity as this affects its susceptibility for cellulase action (Yang et al., 2011). In addition, the presence of residual hemicellulose and lignin influence cellulose digestibility (Yang and Wyman, 2006). Lignin is a known inhibitor for cellulose breakdown by cellulases as it not only influences cellulose accessibility, but also reduces cellulose action by irreversible cellulase adsorption (Yang and Wyman, 2006). Hemicellulases (xylanases and arabinofuranosidases accompanied by glucuronidases and feruloyl and acetyl esterases) are hence necessary to further hydrolyze the hemicelluloses, if one wants to generate additional fermentable sugars and increase cellulose digestibility. The pulp contained also 1.6% of bound acetic acid, which has been reported to decrease enzymatic cellulose digestibility (Pan et al., 2006). The potential of utilization of the obtained cellulose pulp is explored by investigating the production of ethanol, ethylene, isopropanol and organic acids (e.g. itaconic, fumaric and glucaric acid) using biotechnology.

Organosolv lignin typically has good quality as it has high purity (in this case 78%) and consists of relatively low molecular weight lignin (Gosselink et al., 2004). In addition, its polydispersity is generally lower when compared to lignins from other fractionation processes (Xu et al., 2006). Valorization of the lignin fraction is studied by assessment of this fraction for production of functionalized lignins, oligomeric and monomeric phenols. The monomeric phenols include several high-value phenols that might find applications in the pharmaceutical, food and fragrance industrial sectors. In addition, the monomeric phenols can be used as potential anti-knocking additives for transportation fuels. Within BIOCORE the use of oligomeric phenols as well as functionalized lignins is studied in the following applications: manufacture of bio-based polyesters, composite resins, polyurethane foams and coatings as well as for the synthesis of bio-based thermosetting resins for the production of wood panels.

The hemicellulose-rich fraction contained carbohydrates, mainly xylose (both monomeric as oligomeric), which are highly substituted with acetic and uronic acid. This high degree of substitution will impact further processing as enzymatic degradation/fermentation is known to be hindered by substitution of the xylan backbone (Kormelink and Voragen, 1993). Furthermore, several other components that can inhibit enzymatic hydrolysis or microbial fermentation, such as carboxylic acids and phenolic compounds (Thomsen et al., 2009), were present in considerable concentrations in the hemicellulose-rich fraction. Furthermore, it is plausible that degradation products like furfural and hydroxymethylfurfural, also considered as inhibitory compounds (Thomsen et al., 2009), were formed as well, as a small fraction of sugars (7%) was not recovered. Indeed, furfural is a typical degradation product of acid based sugar hydrolysis and will typically be recovered in the aqueous hemicellulose-rich stream of organosolv processes (Kumar et al., 2009). At least partial removal of the above mentioned inhibitors will be necessary when further subjecting this fraction to biochemical conversion. Several approaches can be used for the removal of the above mentioned inhibitors, including ion exchange treatment (Zhuang et al., 2009) and membrane-processes, e.g. ultrafiltration, nanofiltration and electrodialysis (Egüés et al., 2012). As possible applications of the hemicellulose-rich fraction consist of enzymatic or fermentative conversion of xylose, enzymes that could be used to further degrade the highly substituted xylo-oligosaccharides to xylose are xylosidases and reducing-end xylose-releasing exo-oligoxylanases in combination with glucuronidases and feruloyl and acetyl esterases. The hemicelluloses-rich fraction is studied for use in the production of ethanol, ethylene, xylitol, organic acids (e.g. xylonic acid) and alkylpolypentosides. Next to biochemical upgrading, the hemicellulose-rich stream can also be valorized by (thermo)chemical conversion towards furfural.

4. Conclusion

The lab-scale acetic and formic acid based organosolv process proved successful in fractionating wheat straw into its major components cellulose, lignin and hemicellulose. Most xylan (67%) and lignin (96%) were solubilized, generating cellulose pulp with 93% of initial cellulose and purity of 63%. Furthermore, 84% of wheat straw lignin was recovered as lignin with a purity of 78%. The hemicellulose-rich fraction was rich in sugars (58%), but also contained considerable amounts of minerals, proteins and organic acids. Additional process steps, implemented at pilot scale, make it possible to provide cellulose pulp and lignin with higher purity from the discussed process.

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