



Investigation of an effective acid pre-treatment method for the valorisation of Canola fines

A. Ranjan¹ · P. J. Welz¹ · T. Mthethwa¹

Received: 2 December 2022 / Revised: 8 February 2023 / Accepted: 12 February 2023 / Published online: 23 February 2023
© The Author(s) 2023

Abstract

Canola fines are the straw-like material and other residues removed from incoming Canola crops before processing for seed oil. In South Africa, this lignocellulosic biomass is abundant and non-seasonal, making it an ideal substrate for recovery of sugars for valorization to higher value products. Pre-treatment for release of sugars from lignocellulose should be optimized for a given substrate. In this study, mechanical pre-treatment to reduce the size of Canola fines was followed by: (i) steam-assisted acid pre-treatment (0–2% H_2SO_4 % v/v) at different temperatures (20–55 °C), and biomass loading rates (2–8% w/v), and (ii) enzyme hydrolysis, using a central composite experimental design approach. The sugars in the hydrolysates obtained from stages (i) and (ii) were measured quantitatively and qualitatively as outputs that were used to assess process efficiency and to obtain predictive models. The raw and spent Canola fines from each phase were fully characterized in terms of chemical and structural changes at each stage of pre-treatment.

The Canola fines contained 23.0% w/w lignin, 21.4% w/w cellulose, and 15.9% w/w hemicellulose. The maximum yield of total sugars after steam-assisted acid hydrolysis (18.6% w/w) was increased after enzyme hydrolysis (28.3 % w/w) and was higher than reported for other studies pre-treating rapeseed and Canola straws (10.2–11.8% w/w). The sugar profiles in the hydrolysates after steam-assisted acid hydrolysis (xylose>arabinose>glucose) differed from those obtained after acid-assisted enzyme hydrolysis (xylose>glucose>cellobiose>arabinose). The microscopic and chemical analyses of the Canola fines showed sequential breakdown of the lignin, cellulose, and hemicellulose, with some lignin still intact at the end of the pre-treatment. For application of this technology, no heating would be required for acid pre-treatment as temperature was not a significant variable. In addition, the optimal H_2SO_4 concentration was low (1.7% v/v), minimizing the use of hazardous chemicals as well as the risk of releasing microbial and/or enzyme inhibitors during the process. Enzyme hydrolysis should only be required if the downstream application requires higher sugar concentrations and/or different sugar profiles.

Keywords Canola fines · Pre-treatment · Enzyme hydrolysis · Total sugar · Sugar yield · Lignocellulose

Abbreviations

AAEH	Acid-assisted enzyme hydrolysis
CF	Canola fines
LCB	Lignocellulosic biomass
SAAH	Steam-assisted acid hydrolysis
SE	Steam explosion

1 Introduction

Lignocellulosic biomass (LCB) is a readily available, inexpensive, and renewable feedstock for fermentation to biofuels and other value-added products [1]. A variety of plant/agricultural residues have been identified as potential feedstocks for valorisation. These include, among others, sugar beet pulp, pinewood needles, sugarcane bagasse, sorghum bagasse, and rice straw [2–6]. The component ratios of LCB vary from crop-to-crop. Typical values range from 35–45% cellulose, 25–30% hemicellulose, and 15–30% lignin [7]. Lignocellulose is recalcitrant in nature and requires costly pre-treatment to release substrates for bioprocessing. Currently, biofuels produced from LCB cannot compete economically with those from conventional feedstocks such as sugar cane (bioethanol) and oilseeds (biodiesel), despite

✉ A. Ranjan
ranjana@cput.ac.za

¹ Applied Microbial and Health Biotechnology Institute,
Cape Peninsula University of Technology, Symphony way,
Bellville, Cape Town 7535, South Africa

extensive research in this field [8]. Other biorefinery products from LCB with potentially higher market values include biochemicals (e.g., butanol, acetone) and polyhydroxyalkanoate (PHA) precursors for bioplastics [9]. Pre-treatment and hydrolysis increases the porosity of LCB, removes lignin, and reduces cellulosic crystallinity, resulting in the release of the simpler soluble sugars such as glucose, xylose, and arabinose into the hydrolysate for downstream bioprocessing [10].

The most effective pre-treatment of LCB requires a combination of physical, chemical, and hydrolytic reactions. Ideal treatment options should result in the highest sugar yields with minimal release of inhibitors and lowest energy inputs [11]. The first pre-treatment step increases the surface area of the LCB by physically cutting, milling and/or grinding the LCB, usually followed by exposure to high temperature and pressure via steam explosion (SE) to increase the porosity of the feedstock [12]. Chemical pre-treatment relies on acids such as dilute sulfuric acid (dil. H_2SO_4), ammonia/ammonium ($\text{NH}_3/\text{NH}_4^+$), or alkalis such as sodium hydroxide (NaOH) to delignify the LCB and reduce the crystallinity and polymerization of cellulose [13]. While chemical pre-treatment enhances the biodegradability of LCB, higher chemical concentrations may result in the release of microbial inhibitors such as furfurals, salts, and phenolic compounds which may negatively affect the efficiency of downstream biocatalytic reactions [14, 15]. Following chemical pre-treatment, sugar release is often enzymatically enhanced by application of glycoside hydrolases (most commonly cellulases) and lytic polysaccharide monooxygenases [16, 17].

Canola (Canadian oil, low acid) is a rapeseed cultivar originally from Canada that contains negligible erucic acid, a monounsaturated omega-9 fatty acid that is potentially toxic when consumed over long periods [18]. Canola oil has become popular as a healthier alternative to other seed oils, and commercial Canola seed production in South Africa (SA) has increased from 14 to 131452 metric tons between 1999 and 2021 ((South African Grain Industry (SAGIS) [19]). Among other organic agricultural residues, Canola seed processing residues can be considered as feedstocks for valorization into higher value products [20, 21]. During primary and secondary sorting, straw-like LCB residues and other debris known as Canola fines (CF) are separated from the seeds. In SA, Canola crops are silaged upon receipt and then processed over the entire year. The CF and other residues and by-products are therefore non-seasonal in nature, which is a desirable trait when considering downstream usage [21].

This study focussed on finding a simple, effective process for pre-treating CF for maximum release of sugars for downstream fermentation such as the production of PHA or speciality chemicals. Batches of CF were characterized, and pre-treatment was optimized using experiments based on central composite design (CCD) with three factors under

consideration viz, temperature, biomass loading, and chemical loading with dil. H_2SO_4 . Physical and chemical (acid) pre-treatment was followed by enzymatic hydrolysis with a commercial cellulase cocktail. Results were assessed by qualitative and quantitative analysis of the sugars released per unit biomass and structural changes in the CF was studied using scanning electron microscopy (SEM), SEM energy dispersive X-ray (SEM-EDX), powder X-ray diffraction (PXRD), and Fourier transform infrared spectroscopy (FTIR).

2 Materials and method

2.1 Sample collection

A batch of CF was collected from a large edible oil processing facility in SA. The samples consisted of fines from an undetermined mixture of Canola cultivars grown in the Western Cape of SA which include Varola 44, 50, and 54, Monty, Insignia, Scoop, Hylite 200 TT, Hyola 60, Oscar, and Rainbow. The material consisted of all matter taken out by the sieves after offloading and prior to pressing of the seed.

2.2 Characterization of biomass

The chemical composition of CF was determined according to the Laboratory Analytical Procedure (LAP) described by the National Renewable Energy Laboratory (NREL) [22]. This involved analytical acid hydrolysis of 300 mg of test sample followed by autoclaving and incubation in 4% H_2SO_4 in a closed system. The LAP involves standard preparations, neutralization, HPLC, and acetyl analysis. According to the method, each analysis was conducted in quadruplet.

2.3 Pre-treatment and hydrolysis of Canola fines

2.3.1 Physico-mechanical pre-treatment

To reduce the size and increase the area of the CF, they were manually cut into 19 mm then 4.75 mm lengths. Thereafter, they were sieved through a 2.36 mm sieve and allowed to dry overnight in a hot air oven at 50 °C. The dried biomass (pan) <2.36 mm was then ground using a 1200 W Bennett Read extractor (Model: WBL-001H, P.R.C.). Samples of CF after the various mechanical preparation steps are shown in Fig. 1.

2.3.2 Physico-chemical pre-treatment and enzymatic hydrolysis

The pre-treatment of CF was carried out in 250 mL Erlenmeyer flasks, with a working volume of 100 mL. The biomass content and the acid strength were kept



Fig. 1 Samples of Canola fines after sequential physico-mechanical size reductions

as per the DOE (Table 1). Flasks were continuously agitated on an orbital shaker at 150 rpm. Pre-treatment was performed in three sequential stages: (a) chemical hydrolysis, which involves acid pre-treatment of CF with dil. H_2SO_4 for 24 h at 55 °C, (b) SAAH (steam-assisted acid hydrolysis) where, acid treated CF were further steam exploded (SE) at 15 psi pressure and 120 °C temperature for 15 min, and finally (c) AAEH (acid-assisted enzyme hydrolysis): where SAAH was further hydrolysed enzymatically. Prior to enzymatic hydrolysis, the pH of the reaction mixture was adjusted to pH 6.0–7.0 using NaOH pellets/1M H_2SO_4 . Commercial cellulases, namely cellulase from *Trichoderma reesei* (cat no: C2730, Sigma-Aldrich, St. Louis, USA), and Viscozyme®, a cellulolytic enzyme mixture from *Aspergillus* sp. (cat no: V2021, Sigma-Aldrich) were added to the flasks at concentrations of 2 µl/ml each. The flasks were then incubated in a shaking incubator at 150 rpm for 24 h at 50 °C. After each stage, sample aliquots were drawn for quantitative and qualitative analysis of sugars (Section 2.5). The aliquots were centrifuged at 6000 rpm for 10 min, filtered using 0.45 µm sterile filters and stored at −20 °C until required.

2.4 Experimental design

After preliminary testing to inform the levels for each factor (data not shown), the process was optimized in terms of 3 factors: temperature (25–55 °C), biomass loading (2 to 8% weight/volume (w/v)) and chemical loading (0 to 2% dil. H_2SO_4 volume/volume (v/v)) based on central composite design (CCD) using the Design Expert® software version 13 (Stat-Ease, Inc. Minneapolis, USA). Each numeric factor was set to 3 levels: plus and minus 1 (factorial points) and the centre point. The 20 experimental runs that were generated by the software (Table 1) were each performed in duplicate.

2.5 Analytical methods

2.5.1 High-performance liquid chromatography

The soluble sugars in the filtrates (Section 2.3) were identified and quantified using high pressure liquid chromatography (HPLC) with a Phenomenex (Torrance, USA) Rezex RHM monosaccharide H_p (8% cross-linkage) column and an Agilent Technologies 1100 series instrument (Santa Clara, USA) as previously described [23].

Table 1 Statistical design of experiment with three factors studied at three levels

Run	Temp. (°C)	H_2SO_4 (% v/v)	Biomass (% w/v)	Run	Temp. (°C)	H_2SO_4 (% v/v)	Biomass (% w/v)
1	55	1	5	11	40	2	5
2	40	0	5	12	25	0	8
3	40	1	8	13	55	2	8
4	40	1	2	14	25	1	5
5	40	1	5	15	25	0	2
6	25	2	2	16	55	2	2
7	25	2	8	17	40	1	5
8	40	1	5	18	55	0	8
9	40	1	5	19	55	0	2
10	40	1	5	20	40	1	5

2.5.2 Scanning electron microscopy and SEM energy-dispersive X-ray spectroscopy

The SEM images and EDX were obtained using a Zeiss (Oberkochen, Germany) MERLIN Field SEM microscope. Prior to imaging, the samples were mounted on aluminum stubs with double-sided carbon tape. The samples were then coated with a thin (~10 nm thick) layer of gold, using a Leica (Wetzlar, Germany) EM ACE200 Gold sputter coater. The beam conditions during the quantitative analysis and the backscattered electron image analysis were set at 20 kV accelerating voltage, 1.4 nA probe current, 9.5 mm working distance, and a beam current of 11 nA. For the EDX, the X-ray counts and energies were quantified using the Oxford (Abingdon, United Kingdom) Aztec software.

2.5.3 Powder X-ray diffraction

Powder X-ray diffraction (PXRD) analysis was performed using a Bruker D8 advanced diffractometer using CuK α 1 radiation ($\lambda = 1.5418 \text{ \AA}$). The diffractograms were recorded in the range between 5° and 50° of diffraction angle (2θ). A step size of 0.05° was used with 1-s accumulation time. The crystallinity index (CrI) of raw and pre-treated samples was measured using the Segal method (Segal et al., 1959). In this method, the crystalline contribution is determined by the intensity of the 002 peak at $2\theta = 22.6^\circ$ and the amorphous by the intensity at $2\theta = 16.2^\circ$ according to Eq. 1.

$$rI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the maximum intensity of the (002) lattice diffraction peak and I_{am} is the intensity scattered by the amorphous part of the sample.

2.5.4 Fourier transform infrared spectroscopy

FTIR spectrum was recorded between 4000 and 500 cm^{-1} using a Perkin Elmer 1000 series Fourier transform infrared spectrometer with detector at 4 cm^{-1} resolution and 25 scan per sample in the transmission mode.

3 Results and discussion

3.1 Characterization of biomass

The major biopolymer components of CF are cellulose, lignin, and hemicellulose, with minor quantities of proteins, pectins, starch, and fatty acids [16, 24]. Lignin from rapeseed is composed of guaiacyl and syringyl aromatics commonly found in hardwoods and cellulosic fibre lengths

are also similar to those found in hardwoods [25]. In terms of hemicelluloses, xylans typically dominate in hardwoods, while glucomannans dominate in softwoods [8]. Lignin in hardwood is less thermally stable than softwood lignin [8], which is an advantage in terms of pre-treatment using heat to break down lignin.

For biorefinery applications based on fermentation of sugars, pre-treatment of CF to release soluble sugars is required because of the relatively high lignin and hemicellulose contents. In this study, the major macromolecular structural components in the raw CF (%weight/weight (w/w)) were quantified, mainly to assist with the selection of the most suitable pre-treatment method/s. Lignin was present in the highest amount, followed by cellulose and hemicellulose (Fig. 2). Extractives, which include non-structural components of lignocellulose such as fats, phenolics, resin acid, pigments, and waxes constituted $30.5\% \pm 0.06\%$ w/w of the remaining biomass.

The characterization results were compared with those from previous studies where Canola and rapeseed straws/fines were characterized (Table 2). The high variability, especially in lignin content, indicates that the biomass from each location needs to be characterized separately, *viz.* results should not be extrapolated from one study to the next. While some variation may be due to methodological inconsistencies, it is more likely to be associated with differences in plant varieties, climate, and/or the nutritional and chemical status of the terrain [18]. For example, Canola plants tend to accumulate reducing sugars in their stems and leaves which act as the major source of carbon (C) for transformation to fatty acids in the (oil)seeds [26, 27]. While Canola crop yields are enhanced, translocation of fatty acids from the stems to the seeds is disrupted in nitrogen (N)-rich environments. This has a negative effect on oil yield and elevates the sugar content of the stems and leaves, and by inference, the straw [28].

In this study, the lignin content of the raw CF fines was similar to a previous study (Table 2) where the final lignin and cellulose contents were $<1\%$ and 81.4% w/w after alkaline

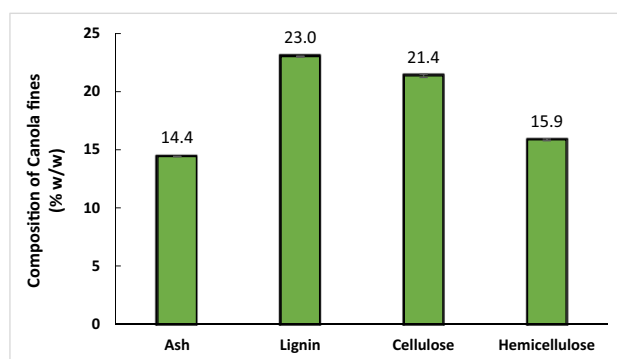


Fig. 2 Macromolecular composition and ash measured in raw Canola fines

Table 2 Literature comparison of lignin, cellulose, and hemicellulose fractions of raw Canola and rapeseed straw/fines

Biomass	Lignin (% w/w)	Cellulose (% w/w)	Hemicellulose (% w/w)	Reference
Canola fines	23.02 ± 0.04	21.36 ± 0.14	15.86 ± 0.08	This study
Rapeseed straw	37.00	31.00	21.00	[25]
Rapeseed straw	25.43 ± 0.11	33.12 ± 1.45	19.71 ± 0.58	[36]
Rapeseed straw	15.55 ± 0.44	36.59 ± 0.82	24.11 ± 1.43	[31]
Rapeseed straw	26.40 ± 1.9	37.80 ± 0.7	-	[32]
Canola straw	19.6 ± 0.6	22.4 ± 0.8	16.9 ± 0.5	[37]
Canola straw	24.3 ± 1.6	38.5 ± 1.5a	24.3 ± 1.6	[29]

pre-treatment with 15% NaOH [29]. In a similar study, a cellulose concentration of 79.7% w/w was achieved by pre-treating raw CF with 0.75% NaOH for 6 h [30]. These studies indicate that pre-treatment of CF is needed to release cellulose from lignin and hemicellulose. Pre-treatment with acids or alkaline solutions are the most widely used and cost-effective methods. If high chemical concentrations and/or temperatures are employed, degradation products such as furfural and 5-hydroxymethylfurfural are released in concentrations that may inhibit downstream microbial and/or enzymatic processes and reduce product yields [31–33]. For environmental sustainability, and to reduce the generation of inhibitors, low concentrations of chemicals are recommended for pre-treatment [34, 35].

This study therefore focussed on maximizing the recovery of soluble sugars from CF with minimal chemical usage. This was achieved by limiting the concentration of H₂SO₄ to a maximum of 2% v/v and enhancing the efficiency of the process by incorporating SE and enzymatic saccharification. While it has been shown that there is an inverse relationship between the size of Canola straw and pre-treatment efficiency [30], this was not a factor to consider in this study as the size of the straw was mechanically reduced (Section 2.3).

3.2 The effects of biomass loading, acid concentration, and temperature on sugar yield

The total sugar (TS) yields (% w/w) were determined from the sum of the sugars identified and quantified in the hydrolysates. In this manuscript, the combination of SE and acid hydrolysis is defined as steam-assisted acid hydrolysis (SAAH) and the combination of SAAH and downstream enzyme hydrolysis is defined as acid-assisted enzyme hydrolysis (AAEH). The results obtained after SAAH and AA EH are presented and discussed separately and compared with one another.

3.2.1 Steam-assisted acid hydrolysis of Canola fines

A general quadratic polynomial with sequential sum of squares for two-factor interactions was used to model the total sugar yields from SAAH of the CF (Table 3). The variation between the factor means viz., temperature (A),

acid concentration (B), and biomass loading (C) relative to the variation within the factors generated a higher *F* statistic and lower *p*-value, thereby rejecting the null hypothesis of the analysis of variance (ANOVA) and concluding the model to be statistically valid. For the fitted model, the *F*-value and *p*-values of 38.42 and <0.0001, respectively, indicated that there was only 0.01% chance that the higher *F*-value was due to noise. The lack of fit (0.61, *p*>0.05) indicated that the model is a moderate to good predictor of TS yield. However, the acid concentration was the only factor that had a significant direct effect on sugar yield (*p* <0.0001).

The predicted *R*² of 0.7678 for the model was in reasonable agreement with the adjusted *R*² of 0.9466 (difference < less than 0.2), while the adequate precision ratio of 16.944, used to measure signal to noise was greater than the desired value of 4. Thus, the model (Eq. 2) can be applied to navigate the design space and predict the TS yield under the optimized set of conditions.

Table 3 Analysis of variance for reduced quadratic model for sugar yield from steam-assisted acid hydrolysis of Canola fines

Source	Sum of squares	Df	Mean square	<i>F</i> -value	<i>p</i> -value
Model	37.01	9	4.11	38.42	< 0.0001
A-Temp	0.0501	1	0.0501	0.4683	0.5093
B-Conc	28.74	1	28.74	268.57	< 0.0001
C-Loading	0.0126	1	0.0126	0.1181	0.7382
AB	0.0478	1	0.0478	0.4467	0.5190
AC	0.0186	1	0.0186	0.1738	0.6855
BC	0.2676	1	0.2676	2.50	0.1449
<i>A</i> ²	0.3222	1	0.3222	3.01	0.1134
<i>B</i> ²	5.05	1	5.05	47.16	< 0.0001
<i>C</i> ²	0.0478	1	0.0478	0.4465	0.5191
Residual	0.1070	10	0.0107		
Lack of fit	0.0406	5	0.0081	0.6113	0.6989
Pure error	0.0664	5	0.0133		
Cor total	3.81	19			

$$TS \text{ yield } (Y) = 3.36 - 0.07A + 1.7B + 0.04C - 0.08AB + 0.05AC + 0.18BC + 0.34A^2 - 1.35B^2 - 0.13C^2 \quad (2)$$

The negligible effect of temperature and biomass loading and highly significant effect of acid concentration on TS yield determined using ANOVA can be seen visually in (Fig. 3). The optimal H_2SO_4 concentration range determined using RSM for SAAH of CF is between 1.5 and 2.0% v/v (Fig. 3).

The sugars in lignocellulosic biomass emanate from the cellulosic and hemicellulosic fractions that can be physically, chemically, and enzymatically broken down into their respective monomeric units [11]. Xylose and arabinose are the aldo-pentoses derived from the hydrolysis of hemicellulose, while glucose typically originates from the breakdown of cellulose [20, 38]. It has previously been shown that acid hydrolysis of rapeseed results in dissolution of glucan and xylans from hemicellulose into glucose and xylose, respectively [39]. In this study, the maximum TS

yield of 18.57% w/w was achieved with a chemical loading of 2.0% v/v H_2SO_4 and biomass loading of 8.0% w/v at 25 °C (Fig. 4). Xylose > arabinose > glucose were the major soluble sugars recovered from SAAH, and only negligible amounts of sugars were released from the experiments conducted with no added H_2SO_4 (Fig. 3). These results indicate that SAAH mainly triggered the breakdown of hemicellulose from the CF.

At all biomass loading rates, there was an increase in TS yield when the H_2SO_4 concentration was increased from 0 to 1% v/v and 1 to 2% v/v, validating the ANOVA results. Biomass loading appeared to have a positive effect on TS yield when increased from 2 to 5% w/v, but not between 5 and 8% w/v. When the biomass loading was increased from 5 to 8% w/v at 1% v/v H_2SO_4 , there was a decreased yield of TS, particularly xylose. This strongly suggests that as the biomass loading increased, there was insufficient acid for efficient hydrolysis at this low (1% v/v) H_2SO_4 concentration. Although the TS yields at 2%

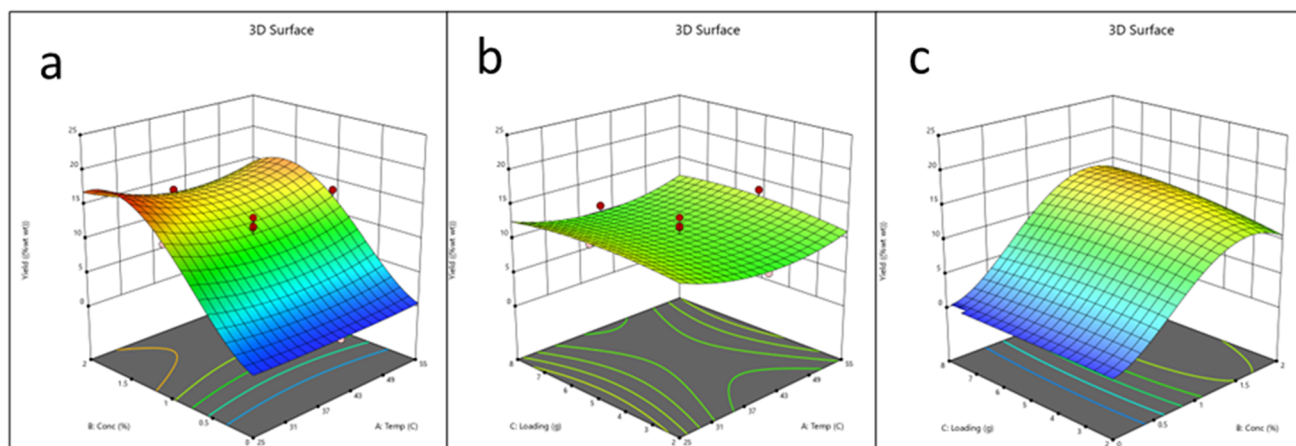
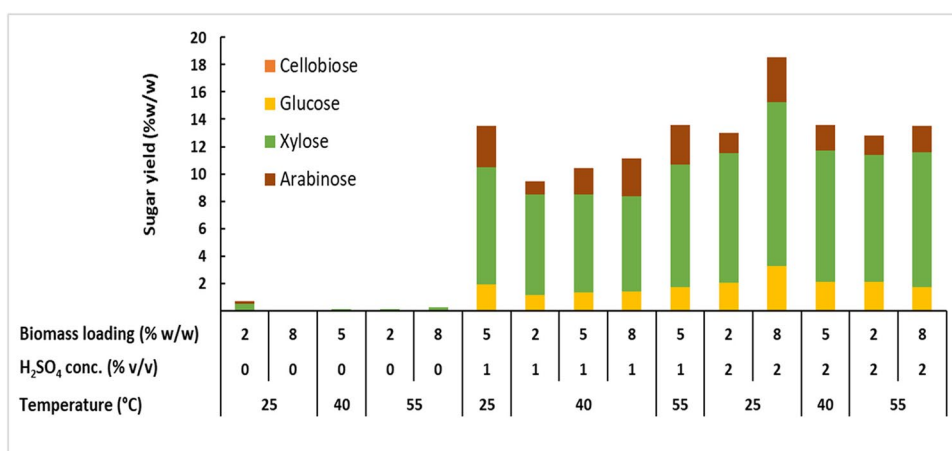


Fig 3 3D response surface plots showing the effects and inter-relationships of factors on total sugar yield: temperature and H_2SO_4 concentration (a), temperature and biomass loading (b), and biomass loading and H_2SO_4 concentration (c)

Fig. 4 Sugars measured in the hydrolysates after steam-assisted acid hydrolysis of Canola fines



v/v H₂SO₄ were similar for biomass loading rates of 5% and 8% w/v (Fig. 4), the actual concentration of the sugars in the hydrolysate would be higher at 8% w/v. As the cost of the substrate (waste CF) is not a factor, in a “real-world” scenario, it may be desirable to apply the higher loading rate to achieve higher sugar concentrations without having to increase the volumetric capacity of the reactor. No temperature dependant trends in sugar yields were noted. This was a positive finding because SAAH of CF could conceivably be conducted at ambient temperatures, obviating the need for heating and preventing the release of inhibitors for any downstream microbial and/or enzymatic processes [32, 33]

The maximum TS yield of 18.5 % w/w from SAAH was notably higher than reported in previous studies reporting on hydrolysis of Canola residues. Fermentable sugar yields of 10.2% w/w and 11.5% w/w were obtained from subcritical water hydrolysis of Canola silique and stalks, respectively [40], and a TS yield of 11.8% w/w consisting of 2.5% w/w xylose and 9.3% w/w glucose were obtained using similar methodology (SAAH) to this study [41]. In a third study, the authors [32] did not report on the TS yield, only the sugar concentration (7.22 g/L) after hydrothermal and acid treatment of rapeseed straw. However, the authors also reported dominance of pentose sugars, in keeping with the results of this study. Overall, the results indicated that the SAAH method applied is a suitable pre-treatment method for obtaining high TS yields.

3.2.2 Acid-assisted enzyme hydrolysis of Canola fines

A polynomial quadratic model was obtained from ANOVA of the AAEH data using the TS yield as the response and H₂SO₄ concentration and biomass loading as input factors. Temperature did not have a significant effect on TS yield after SAAH, and all the AAEH runs were conducted at 40 °C. Temperature was therefore discounted as a factor for analysis of the AAEH results. A high *F*-value (20.61) and low *p*-value (<0.0001) were obtained, with only a 0.01% chance that the *F*-value obtained was due to noise. The null hypothesis was therefore rejected, and it was concluded that the model was valid. As with the SAAH, biomass loading (2 to 5% w/v) was insignificant as a stand-alone factor, while H₂SO₄ concentration (0 to 2% v/v) was highly significant. However, the model showed that both factors (BC, Table 4) were significant when combined.

The predicted *R*² of 0.6802 for the model was in reasonable agreement with the Adjusted *R*² of 0.8377, with an adequate signal to noise ratio. Akaike's information criterion (AIC) of 47.58 and Bayesian Information Criterion (BIC) of 47.09 showed the quadratic model to be a best fit. The model may be used to predict the TS yield from AAEH at different H₂SO₄ concentrations and biomass loading rates

Table 4 Analysis of variance for reduced quadratic model for sugar yield from acid-assisted enzyme hydrolysis of Canola fines

Source	Sum of squares	Df	Mean square	<i>F</i> -value	<i>p</i> -value
Model	36.96	5	7.39	20.61	< 0.0001
B-Conc	18.95	1	18.95	52.83	< 0.0001
C-Loading	1.64	1	1.64	4.59	0.0503
BC	2.10	1	2.10	5.86	0.0296
<i>B</i> ²	4.78	1	4.78	13.32	0.0026
<i>C</i> ²	1.33	1	1.33	3.71	0.0748
Residual	5.02	14	0.3587		
Lack of fit	4.61	9	0.5120	6.20	0.0293
Pure error	0.4132	5	0.0826		
Cor total	41.98	19			

within the ranges of the parameters used in the experiments. The quadratic model was adjusted to the experimental data to produce Eq. 3.

$$\text{TS yield (Y)} = 4.39 + 1.38B + 0.41C + 0.51BC - 1.22B^2 - 0.65C^2 \quad (3)$$

The effects of biomass loading and concentration on TS yield can be seen visually in Fig. 5. The optimal (most desirable) position (0.927) for maximum TS yield (25.15% w/w) are shown in Fig 5a and 5b, respectively. From the coordinates, increases in TS yield should be obtained at biomass loading up until the optimal ~6.8% w/v and H₂SO₄ concentration up until the optimal ~1.7%.

Hemicellulases include xylanases, mannases, xylosidases, and arabinofuranosidases [8]. Cellulases and polysaccharide monooxygenases trigger cellulose hydrolysis through oxidative chain breakages. Complete hydrolysis of cellulose results in the release of glucose, but incomplete hydrolysis may result in release of cellobiose which is a disaccharide consisting of two glucose units joined through β-(1,4') glycosidic bonds.

In this study, enzymatic saccharification resulted in additional release of soluble sugars from cellulose and hemicellulose after AAEH of CF. A notable increase in TS yield between SAAH and AAEH was seen for most of the experimental runs (Fig. 6a), with additional release of monosaccharides as well as cellobiose (Fig. 4, Fig. 6b). A maximum TS yield of 28.3% w/w was obtained from AAEH with a biomass loading of 8% w/v and H₂SO₄ concentration of 2% v/v for the preceding SAAH step. Under these conditions, the sugars in the hydrolysate consisted of 12.5% w/w xylose, 7.4% w/w glucose, 5.4% w/w cellobiose, and 3.0% w/w arabinose, with respective increases in (w/w) yields of 2.6%, 4.7%, 5.4%, and 1.1%. The abundance distribution of the sugars changed from xylose>arabinose>glucose after SAAH to xylose>glucose>cellobiose>arabinose after AAEH. As xylans

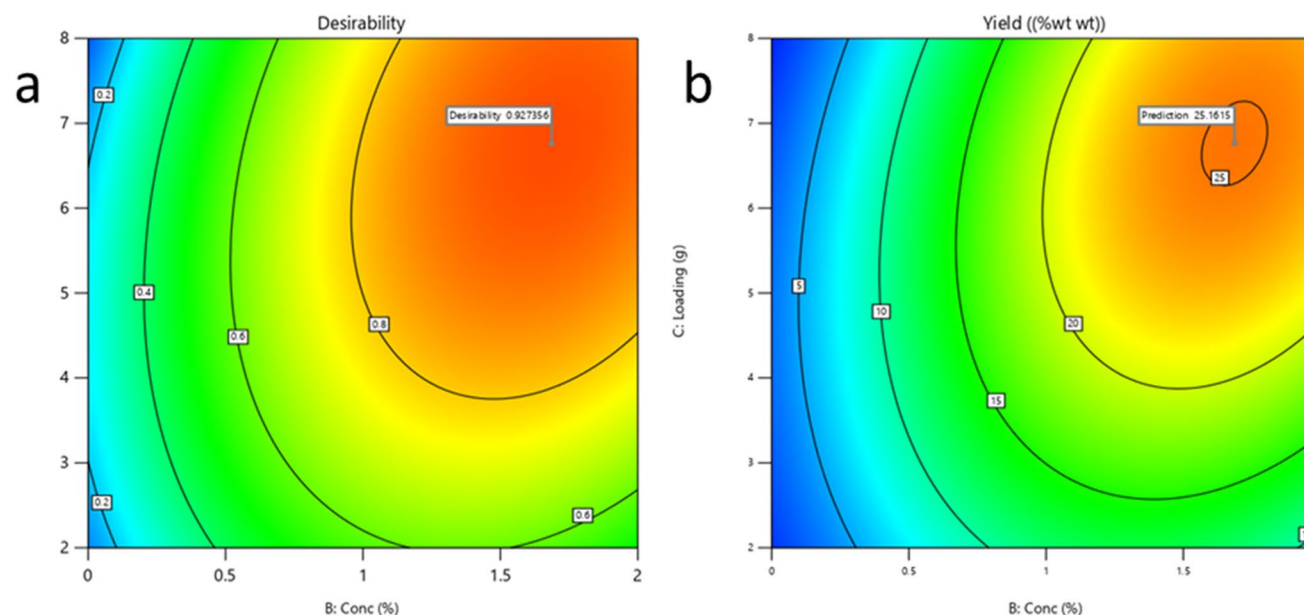


Fig. 5 Contour graphs for desirability **(a)** and total sugar yield **(b)** from acid-assisted enzyme hydrolysis

dominate the make-up of hemicelluloses in hardwoods [8, 42], the high relative xylose content found in the hydrolysates supports previous research where it was shown that the chemical structure of rapeseed straw is more akin to hardwood than softwood [25, 43]. No cellobiose was found in any of the hydrolysates after SAAH (Fig. 4), but was found in all of the AAEH hydrolysates with biomass loading >2% w/v and H_2SO_4 concentrations of 1% and 2% v/v. The most plausible explanation for the absence of cellobiose at low biomass loading (2% w/v) was that there was greater enzyme accessibility with less biomass present, and that any cellobiose that was released was hydrolysed to glucose. This could form a case for increased incubation time (>24 h) and/or the use of reduced biomass loading for the second (AAEH) stage. Indeed, increased incubation may result in additional hydrolysis of cellulose to glucose.

4 Scanning electron microscopy analysis

Scanning electron microscopy was used to study the morphology of the raw and pre-treated CF (Fig. 7). Smooth and continuous surfaces were noted in the images of the raw CF (Fig. 7a, b). Sequential physical structural damage was noted after each pre-treatment step (Fig. 7a–g) as previously described [44, 45], but some fragments were left intact. This indicated that the lignin was not completely degraded, but that the pre-treatment increased the structural surface area available for acid and then enzyme activity for release of sugars [46].

5 Scanning electron microscopy energy-dispersive X-ray spectroscopy analysis

Changes in the elemental composition of the CF were monitored using EDX (Table 5). In all samples, carbon (C) and oxygen (O) were the main atomic elements, and both decreased after each sequential step of pre-treatment. The C content decreased from 62.92% w/w in the raw CF to 48.77% w/w after AAEH, while only minimal decreases in the elemental O occurred (Table 5). Lignin contains high concentrations of C and decreasing C content has been ascribed to delignification of biomass [47]. It was therefore assumed that the pre-treated CF contained less lignin than the raw fines. Furthermore, the decreasing O content could indicate hydrolysis of cellulose and associated release of sugars. The residues of other minor inorganic elements, namely, magnesium (Mg), silica (Si), potassium (K), and calcium (Ca) were also reduced by pre-treatment, suggesting a reduction in ash content (Table 5).

5.1 Powder X-ray diffraction analysis

The CrI provides a measure of the relative fraction of crystalline cellulose in the CF. It is influenced by the presence of the amorphous lignin and hemicelluloses. All the diffractograms from the PXRD analyses of raw and pre-treated CF show the typical XRD peaks of cellulose (Fig. 8). The CrI's of all the pre-treated CF samples were lower than that of the raw CF as reported in Table 6. Decreased CrI values after pre-treatment of LCB have been observed in previous studies [48–50]. The

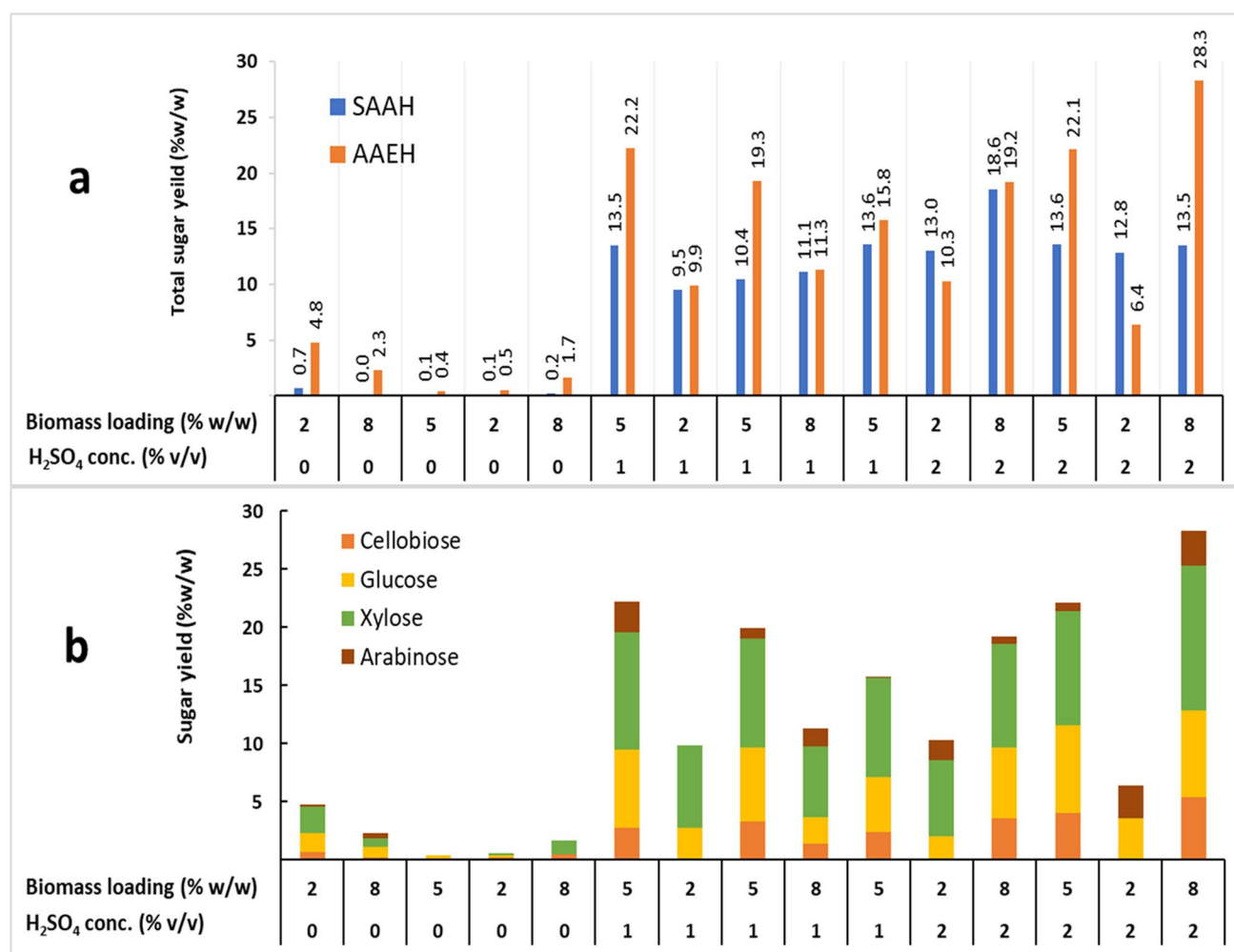


Fig. 6 Comparison of total sugar yields in the hydrolysates obtained from steam-assisted acid hydrolysis (SAAH) and acid-assisted enzyme hydrolysis (AAEH) (a), and sugars measured in the final hydrolysates after AAEH (b)

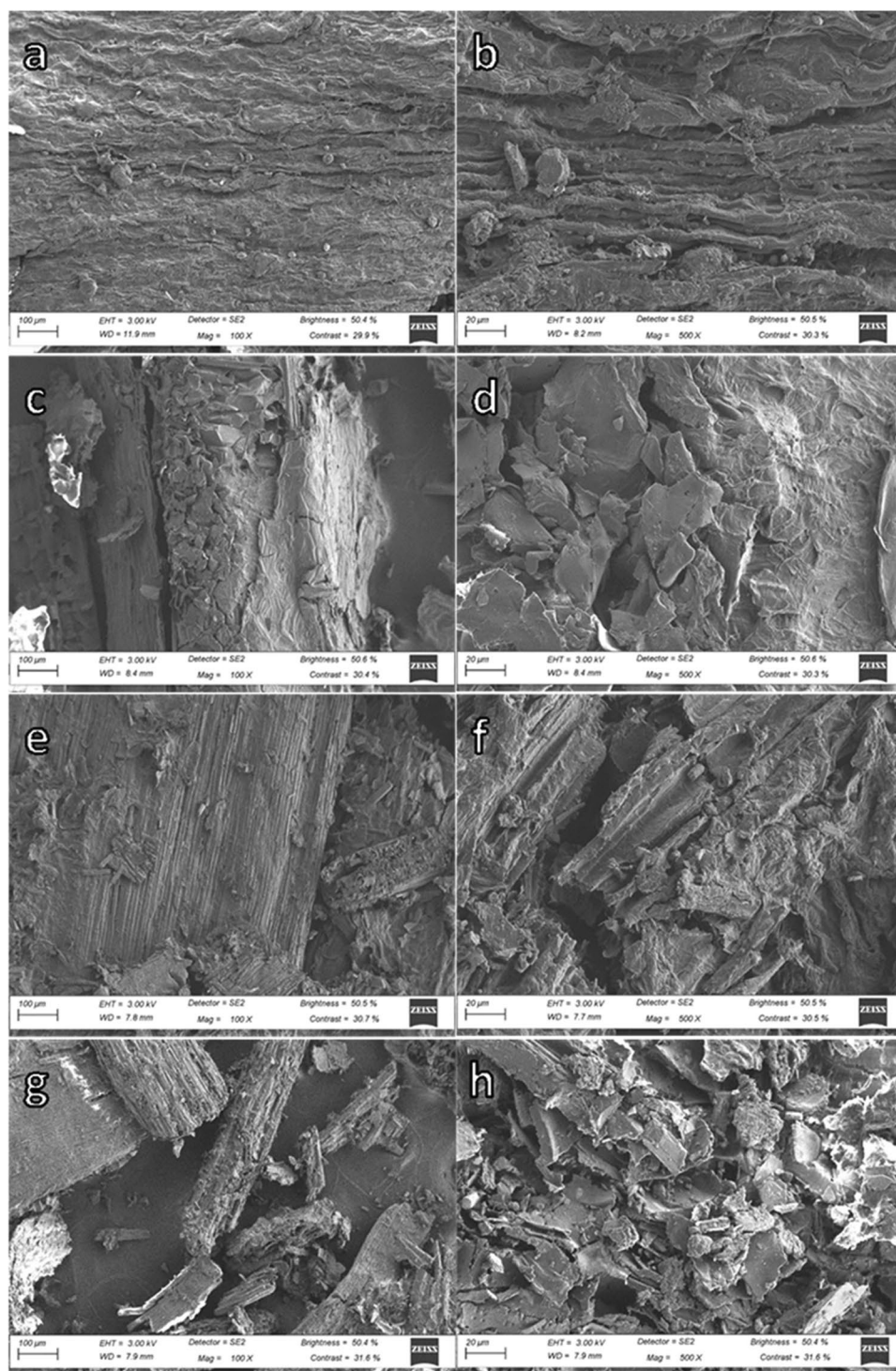
decreasing crystallinity was associated with the disruption of the intra- and inter-molecular hydrogen (H) bonds in the cellulose due to the applied pre-treatment methods which increased the LCB surface area [51]. After the first pre-treatment stages, the CrI decreased from 32.9 % (raw CF) to 15.8 % (after SE) and 13.8% (after SAAH). However, the CrI increased to 21.5 % after AAEH, which is still lower than the CrI of the raw CF. This increase could be attributed to the removal or degradation of a large portion of the amorphous regions of the CF. The presence of sharp peaks following AAEH are due to the crystalline cellulose structure [47].

5.2 Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy was employed to elucidate the effects of pre-treatment on the chemical structures of the CF (Fig. 9). This was achieved by comparing the

infrared (IR) spectra of the raw CF and the CF sampled after each sequential step of pre-treatment. The band detected at 3308 cm⁻¹ of the spectra is associated with the stretching of O–H bonds, which includes both aliphatic and aromatic hydroxyl groups in cellulose, hemicellulose, and lignin structures [52]. Compared to the raw CF, this peak was altered in the pre-treated samples due to the breaking of the ether bonds between the cellulose and lignin resulting in the formation of additional hydroxyl groups. The peaks at 2919 and 2845 cm⁻¹ can be attributed to C–H stretching vibration from the methoxy and methyl groups [53]. The presence of lignin was confirmed by the appearance of stretching vibrations of the aromatic structure at wavenumbers of 1421, 1537, and 1603 cm⁻¹ [54, 55]. After pre-treatment, there was no significant change in these vibrations, which suggests that the main structure of lignin was not completely broken as also evidenced by the SEM images (Fig. 7). The shoulder peak at 1737 cm⁻¹ is associated with the stretching of C=O

Fig. 7 SEM images at $\times 100$ (left) and $\times 500$ (right) magnification of raw Canola fines (a, b), and Canola fines after steam explosion (SE) (c, d), acid-assisted steam explosion (SAAH) (e, f), and acid-assisted enzyme hydrolysis (AAEH) (g, h)



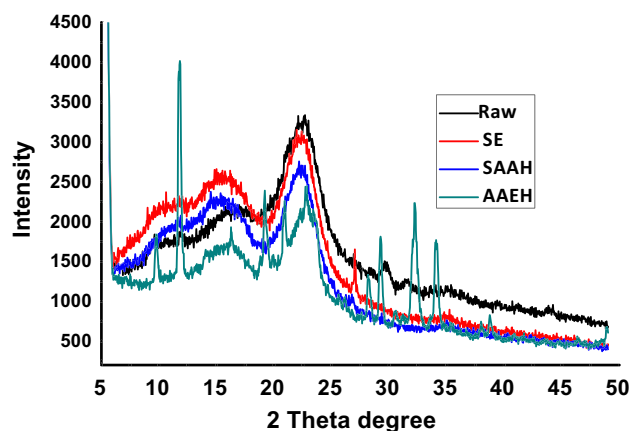
in hemicellulose [56]. The absorption at this wavenumber decreased after pre-treatment, indicating that the linkages in the hemicellulose were broken. The bands observed at 1034 to 1103 cm^{-1} can be attributed to the vibration of C–O and C–C stretch in cellulose, hemicellulose, and lignin [57, 58]. These peaks became more pronounced in the pre-treated samples, which indicated the removal of hemicellulose [56, 59].

A stronger effect was observed after each sequential effect, indicating the effectiveness of the pre-treatment in removing hemicellulose. A decrease in the intensity of the 1034 to 1103 cm^{-1} absorptions following the AAEH pre-treatment is associated with delignification [60, 61]. The peak at 1319 cm^{-1} corresponds to the C–O vibration of the syringyl ring in the structure of lignin [62, 63]. The small peak at the fingerprint

Table 5 Percentage of elements in raw and pre-treated Canola fines detected using SEM-EDX

	Raw CF (% w/w)	Pre-treated CF (% w/w)		
		SE	SAAH	AAEH
C	62.92 ±6.15	56.26 ±15.32	52.19 ±15.39	48.77 ±18.45
O	26.99 ±3.86	25.59 ±4.75	23.83 ±4.75	23.46 ±7.33
Mg	0.54 ±0.20	0.10 ±0.11	0.06 ±0.06	0.03 ±0.13
Si	1.12 ±2.73	0.18 ±0.10	0.07 ±0.07	0.06 ±3.74
K	3.24 ±1.04	0.27 ±4.75	0.23 ±0.17	0.13 ±1.51
Ca	0.97 ±0.33	0.43 ±4.66	0.15 ±3.14	0.12 ±3.20

CF, Canola fines; SE, steam explosion; SAAH, steam-assisted acid hydrolysis; AAEH, acid-assisted enzyme hydrolysis

**Fig. 8** X-ray diffraction patterns of raw canola fines (raw) and canola after steam explosion (SE), steam-assisted acid hydrolysis (SAAH), and acid-assisted enzyme hydrolysis (AAEH)**Table 6** Crystallinity index of raw canola and canola after steam explosion, chemical pre-treatment, and enzyme hydrolysis

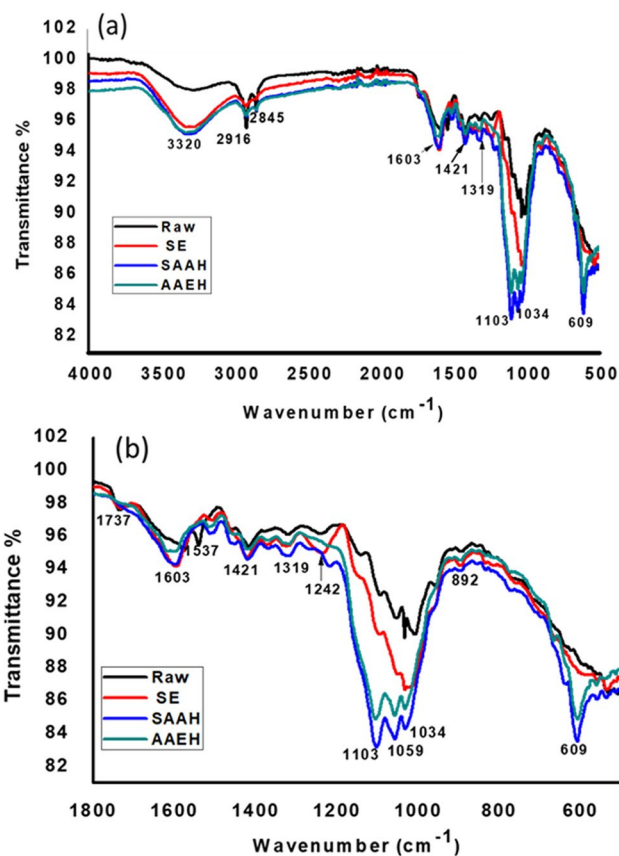
Sample	CrI (%)
Raw	32.9
SE	15.8
SAAH	13.8
AAEH	21.5

CrI, crystallinity index

region at 892 is related to the cellulosic β -glycosidic linkages [62, 64]. The peak at 609 cm^{-1} observed in the pre-treated canola fines is typical of pure cellulose [65].

6 Conclusions and recommendations

In this study, the maximum TS yields in the hydrolysates were 18.6% w/w after SAAH and 28.3% w/w after AAEH. To the best of our knowledge, these values are higher than those obtained in other studies reporting on sugar release

**Fig. 9** Fourier transform infrared spectroscopy spectra of (i) untreated canola fines (raw), (ii) steam exploded (SE), (iii) steam-assisted acid hydrolysis (SAAH), (iv) acid-assisted enzyme hydrolysis (AAEH) at wavenumbers of 500–4000 cm^{-1} (a) and the fingerprint region of 500–1800 cm^{-1} (b)

from Canola or rapeseed straw/fines (10.2–11.8% w/w). The sugar profiles in the hydrolysates after steam-assisted acid hydrolysis (xylose>arabinose>glucose) differed from those obtained after acid-assisted enzyme hydrolysis ((xylose (12.5% w/w) >glucose (7.4% w/w) >cellobiose (5.4% w/w)>arabinose (3.0% w/w)).

For application of this technology, no heating would be required for acid pre-treatment because, within the range tested (20–55°C), temperature was not a significant variable. In addition, the optimal H_2SO_4 concentration was low (1.7% v/v), minimizing the use of hazardous and costly chemicals as well as the risk of releasing microbial and/or enzyme inhibitors during the pre-treatment process. As relatively high concentrations of sugars were obtained from AAEH, this hydrolysate could be used “as is.” If higher concentrations of sugars, or different sugar profiles are required for a particular downstream application, the AAEH could be followed by enzyme hydrolysis, which could be further optimized to obtain even higher sugar concentrations.

Future work will consist of optimizing the growth of microbial species in the hydrolysates for the production of value-added chemicals and compounds.

Author contribution The authors confirm contribution to the manuscript as follows: study conception, design, and data collection: A Ranjan (AR); analysis and interpretation of results: AR, PJ Welz (PJW), Thandekile Mthethwa (TM); manuscript preparation: AR, PJW, TM.

Funding Open access funding provided by Cape Peninsula University of Technology. This project was funded by the Department of Science and Innovation (DSI) of South Africa via the Council for Scientific and Industrial Research (CSIR) Waste Research Development and Innovation (RDI) Roadmap (Grant number CSIR/BEI/WRIU/2020/033). Any opinions, findings and conclusions, or recommendations expressed in this material are those of the authors and the funding entities do not accept any liability in this regard.

Data availability Data will be made available on request.

Declarations

Ethical approval Not applicable. No human and/or animals were used in the study.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Sherif A, Hussen A, Firemichael D (2021) Hydrolysis of multi substrate biomass using para-toluenesulphonic acid for bioethanol production: a promising option over the sulfuric acid treatment. *Biomass Bioenerg* 144:105922. <https://doi.org/10.1016/j.biombioe.2020.105922>
2. Rana AK et al (2022) Sugar beet pulp: resurgence and trailblazing journey towards a circular bioeconomy. *Fuel* 312:122953. <https://doi.org/10.1016/j.fuel.2021.122953>
3. Rana AK, Guleria S, Gupta VK, Thakur VK (2023) Cellulosic pine needles-based biorefinery for a circular bioeconomy. *Bioresour Technol* 367:128255. <https://doi.org/10.1016/j.biortech.2022.128255>
4. Rana AK (2022) Green approaches in the valorization of plant wastes: recent insights and future directions. *Curr Opin Green Sustain Chem* 38:100696. <https://doi.org/10.1016/j.cogsc.2022.100696>
5. Nunta R, Techapun C, Sommanee S et al (2023) Valorization of rice straw, sugarcane bagasse and sweet sorghum bagasse for the production of bioethanol and phenylacetylcarbinol. *Sci Rep* 13:727. <https://doi.org/10.1038/s41598-023-27451-4>
6. Ranjan A, Moholkar VS (2013) Comparative study of various pretreatment techniques for rice straw saccharification for the production of alcoholic biofuels. *Fuel* 112:567–571. <https://doi.org/10.1016/j.fuel.2011.03.030>
7. Raj T, Chandrasekhar K, Naresh Kumar A, Kim SH (2022) Lignocellulosic biomass as renewable feedstock for biodegradable and recyclable plastics production: a sustainable approach. *Renew Sustain Energy Rev* 158:112130. <https://doi.org/10.1016/j.rser.2022.112130>
8. Singhvi MS, Gokhale DV (2019) Lignocellulosic biomass: hurdles and challenges in its valorization. *Appl Microbiol Biotechnol* 103(23–24):9305–9320. <https://doi.org/10.1007/s00253-019-10212-7>
9. Usmani Z et al (2020) Ionic liquid based pretreatment of lignocellulosic biomass for enhanced bioconversion. *Bioresour Technol* 304:123003. <https://doi.org/10.1016/j.biortech.2020.123003>
10. Usmani Z et al (2021) Lignocellulosic biorefineries: the current state of challenges and strategies for efficient commercialization. *Renew Sustain Energy Rev* 148:111258. <https://doi.org/10.1016/j.rser.2021.111258>
11. Alawad I, Ibrahim H (2022) Pretreatment of agricultural lignocellulosic biomass for fermentable sugar: opportunities, challenges, and future trends. *Biomass Convers Biorefinery*. <https://doi.org/10.1007/s13399-022-02981-5>
12. Kumar B, Bhardwaj N, Agrawal K, Chaturvedi V, Verma P (2020) Current perspective on pretreatment technologies using lignocellulosic biomass: an emerging biorefinery concept. *Fuel Process Technol* 199:106224. <https://doi.org/10.1016/j.fuproc.2019.106244>
13. Soltanian S et al (2020) A critical review of the effects of pretreatment methods on the exergetic aspects of lignocellulosic biofuels. *Energy Convers Manag* 212:112792. <https://doi.org/10.1016/j.enconman.2020.112792>
14. Behera S, Arora R, Nandhagopal N, Kumar S (2014) Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. *Renew Sustain Energy Rev* 36:91–106. <https://doi.org/10.1016/j.rser.2014.04.047>
15. Ríos-González LJ, Medina-Morales MA, Rodríguez-De la Garza JA, Romero-Galarza A, Medina DD, Morales-Martínez TK (2020) Comparison of dilute acid pretreatment of agave assisted by microwave versus ultrasound to enhance enzymatic hydrolysis. *Bioresour Technol* 319:124099. <https://doi.org/10.1016/j.biortech.2020.124099>
16. Bhatia SK, Jagtap SS, Bedekar AA, Bhatia RK et al (2020) Recent developments in pretreatment technologies on lignocellulosic biomass: effect of key parameters, technological improvements, and challenges. *Bioresour Technol* 300:122724. <https://doi.org/10.1016/j.biortech.2019.122724>
17. Ezeilo UR, Zakaria II, Huyop F, Wahab RA (2017) Enzymatic breakdown of lignocellulosic biomass: the role of glycosyl hydrolases and lytic polysaccharide monooxygenases. *Biotechnol Biotechnol Equip* 31(4):647–662. <https://doi.org/10.1080/13102818.2017.1330124>
18. Eskin NAM (2016) Rapeseed oil/canola. In: Caballero B, Finglas PM, Toldrá F (eds) *Encyclopedia of food and health*, 1st edn. Academic Press, Elsevier, pp 581–585. <https://doi.org/10.1016/B978-0-12-384947-2.00585-7>
19. South African Grain Information Service (SAGIS) information/data (2022) <https://www.sagis.org.za/>. Accessed 26 Nov 2022
20. Dietrich K, Dumont MJ, Del Rio LF, Orsat V (2019) Sustainable PHA production in integrated lignocellulose biorefineries. *N Biotechnol* 49:161–168. <https://doi.org/10.1016/j.nbt.2018.11.004>

21. Welz PJ (2019) Edible seed oil waste: status quo and future perspectives. *Water Sci Technol* 80(11):2107–2116. <https://doi.org/10.2166/wst.2020.043>
22. Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW (2010) Compositional analysis of lignocellulosic feedstocks 1 Review and description of methods. *J Agric Food Chem* 58(16):9043–9053. <https://doi.org/10.1021/jf1008023>
23. Welz PJ, Holtman G, Haldenwang R, Le Roes-Hill M (2016) Characterisation of winery wastewater from continuous flow settling basins and waste stabilisation ponds over the course of 1 year: implications for biological wastewater treatment and land application. *Water Sci Technol* 74(9):2036–2050. <https://doi.org/10.2166/wst.2016.226>
24. Yang C, Lü X (2021) Composition of plant biomass and its impact on pretreatment. In: Lü X (ed) Woodhead publishing series in energy. Advances in 2nd generation of bioethanol production. Woodhead Publishing, pp 71–85. <https://doi.org/10.1016/B978-0-12-818862-0.00002-9>
25. Svärd A (2018) Biopolymers and materials from rapeseed straw biorefining. Doctoral thesis, KTH Royal Institute of Technology, University in Stockholm, Sweden. <http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A1246419&dswid=2869>
26. Khan S et al (2018) Alteration in yield and oil quality traits of winter rapeseed by lodging at different planting density and nitrogen rates. *Sci Rep* 8(1):1–12. <https://doi.org/10.1038/s41598-017-18734-8>
27. Kordrostami M, Mafakheri M (2020) Rapeseed: biology and physiological responses to drought stress. In: Hasanuzzaman M (ed) The plant family brassicaceae. Springer, Singapore, pp 263–276. https://doi.org/10.1007/978-981-15-6345-4_8
28. Ghafoor A et al (2022) Carbohydrates accumulation, oil quality and yield of rapeseed genotypes at different nitrogen rates. *Plant Prod Sci* 25(1):50–69. <https://doi.org/10.1080/1343943X.2021.1943464>
29. Ciftci D, Flores RA, Saldaña MDA (2018) Cellulose fiber isolation and characterization from sweet blue lupin hull and canola straw. *J Polym Environ* 26(7):2773–2781. <https://doi.org/10.1007/s10924-017-1164-5>
30. Agu OS, Tabil LG, Dumonceaux T (2021) Enzymatic saccharification of canola straw and oat hull subjected to microwave-assisted alkali pretreatment. In: Marzouki R (ed) Alkaline chemistry and applications. Intechopen, pp 1–16. <https://doi.org/10.5772/intechopen.96394>
31. Díaz MJ, Cara C, Ruiz E, Romero I, Moya M, Castro E (2010) Hydrothermal pre-treatment of rapeseed straw. *Bioresour Technol* 101(7):2428–2435. <https://doi.org/10.1016/j.biortech.2009.10.085>
32. Jeong TS (2011) Oh KK (2011) Bioresource Technology Optimization of fermentable sugar production from rape straw through hydrothermal acid pretreatment *Bioresour. Technol* 102(19):9261–9266. <https://doi.org/10.1016/j.biortech.2011.06.092>
33. Chundawat SPS, Balan V, Da costa Sousa L, Dale BE (2010) 2 - Thermochemical pretreatment of lignocellulosic biomass. In: Waldron K (ed) Woodhead publishing series in energy. Bioalcohol Production, Woodhead Publishing, pp 24–72. <https://doi.org/10.1533/9781845699611.1.24>
34. Noonari AA, Shah AR, Mirjat NH, Anh T (2022) Various pretreatments of canola straw with hydrogen peroxide, calcium hydroxide, silica, and *Pleurotus ostreatus* to improve methane yield through anaerobic co-digestion. *Biomass Convers Biorefinery*. <https://doi.org/10.1007/s13399-021-02226-x>
35. Tahir MH, Çakman G, Goldfarb JL, Topcu Y, Naqvi SR, Ceylan S (2019) Demonstrating the suitability of canola residue biomass to biofuel conversion via pyrolysis through reaction kinetics, thermodynamics and evolved gas analyses. *Bioresour Technol* 279:67–73. <https://doi.org/10.1016/j.biortech.2019.01.106>
36. Brahim M, Boussetta N, Grimi N, Vorobiev E, Zieger-Devin I, Brosse N (2017) Pretreatment optimization from rapeseed straw and lignin characterization. *Ind Crops Prod* 95:643–650. <https://doi.org/10.1016/j.indcrop.2016.11.033>
37. Adapa PK, Schonenau LG, Canam T, Dumonceaux T (2011) Quantitative analysis of lignocellulosic components of non-treated and steam exploded barley, canola, oat and wheat straw using Fourier transform infrared spectroscopy. *Faculty Research & Creative Activity* 107. http://thekeep.eiu.edu/bio_fac/107
38. Boldrin A, Balzan A, Astrup T (2013) Energy and environmental analysis of a rapeseed biorefinery conversion process. *Biomass Convers Biorefinery* 3(2):127–141. <https://doi.org/10.1007/s13399-013-0071-9>
39. Castro E, Díaz MJ, Cara C, Ruiz E, Romero I, Moya M (2011) Dilute acid pretreatment of rapeseed straw for fermentable sugar generation. *Bioresour Technol* 102(2):1270–1276. <https://doi.org/10.1016/j.biortech.2010.08.057>
40. de Oliveira MB, dos Santos MSN, Pogorzelski ES et al (2022) Potential of canola feedstocks for fermentable sugars production by subcritical water hydrolysis. *Biomass Bioenerg* 162:106505. <https://doi.org/10.1016/j.biombioe.2022.106505>
41. George N, Yang Y, Wang Z, Sharma-Shivappa R, Tungate K (2010) Suitability of canola residue for cellulosic ethanol production. *Energy Fuels* 24(8):4454–4458. <https://doi.org/10.1021/ef1002155>
42. Isikhuemhen OC, Mikiashvili NA, Senwo ZN, Ohimain EI (2014) Biodegradation and sugar release from canola plant biomass by selected white rot fungi. *Adv Biol Chem* 04(06):395–406. <https://doi.org/10.4236/abc.2014.46045>
43. Svärd A, Brännvall E, Edlund U (2017) Rapeseed straw polymeric hemicelluloses obtained by extraction methods based on severity factor. *Ind Crops Prod* 95:305–315. <https://doi.org/10.1016/j.indcrop.2016.10.038>
44. Banerjee D, Mukherjee S, Pal S, Khowala S (2016) Enhanced saccharification efficiency of lignocellulosic biomass of mustard stalk and straw by salt pretreatment. *Ind Crops Prod* 80:42–49. <https://doi.org/10.1016/j.indcrop.2015.10.049>
45. Swain MR, Krishnan C (2015) Improved conversion of rice straw to ethanol and xylitol by combination of moderate temperature ammonia pretreatment and sequential fermentation using *Candida tropicalis*. *Ind Crops Prod* 77:1039–1046. <https://doi.org/10.1016/j.indcrop.2015.10.013>
46. Yang H, Chang Geun Y, Xianzhi M et al (2020) Structural changes of lignins in natural *Populus* variants during different pretreatments. *Bioresour Technol* 295:12224. <https://doi.org/10.1016/j.biortech.2019.122240>
47. Kundu C, Samudrala SP, Kibria MA, Bhattacharya S (2021) One-step peracetic acid pretreatment of hardwood and softwood biomass for platform chemicals production. *Sci Rep* 11(1):1–11. <https://doi.org/10.1038/s41598-021-90667-9>
48. Bali G, Meng X, Deneff JJ, Sun Q, Ragauskas AJ (2015) The effect of alkaline pretreatment methods on cellulose structure and accessibility. *Chem Sus Chem* 8(2):275–279. <https://doi.org/10.1002/cssc.201402752>
49. Dodo CM, Mamphweli S, Okoh O (2017) Bioethanol production from lignocellulosic sugarcane leaves and tops. *J Energ South Africa* 28(3):1–11. <https://doi.org/10.17159/2413-3051/2017/v28i3a2354>
50. Kumari D, Singh R (2022) Rice straw structure changes following green pretreatment with petha wastewater for economically viable bioethanol production. *Sci Rep* 12(1):1–10. <https://doi.org/10.1038/s41598-022-14627-7>
51. Myint AA, Kim DS, Lee HW et al (2013) Impact of bleaching on subcritical water- and Formosolv-pretreated tulip tree to enhance enzyme accessibility. *Bioresour Technol* 145:128–132. <https://doi.org/10.1016/j.biortech.2013.03.069>

52. Rajput AA, Zeshan VC (2018) Effect of thermal pretreatment on chemical composition, physical structure and biogas production kinetics of wheat straw. *J Environ Manage* 221:45–52. <https://doi.org/10.1016/j.jenvman.2018.05.011>
53. Zhuang J, Li M, Pu Y, Ragauskas AJ, Yoo CG (2020) Observation of potential contaminants in processed biomass using fourier transform infrared spectroscopy. *Appl Sci* 10(12):1–13. <https://doi.org/10.3390/app10124345>
54. Lu Y, Lu YC, Hu HQ, Xie FJ, Wei XY (2017) Fan X (2017) Structural characterization of lignin and its degradation products with spectroscopic methods. *J Spectrosc* 8951658:15. <https://doi.org/10.1155/2017/8951658>
55. de Souza LL, Daniel LA (2020) Optimization of microalgae harvesting by sedimentation induced by high pH. *Water Sci Technol* 82(6):1227–1236. <https://doi.org/10.2166/wst.2020.106>
56. Zhang J, Ma X, Yu J, Zhang X, Tan T (2011) The effects of four different pretreatments on enzymatic hydrolysis of sweet sorghum bagasse. *Bioresour Technol* 102(6):4585–4589. <https://doi.org/10.1016/j.biortech.2010.12.093>
57. Xu F, Yu J, Tesso T, Dowell F, Wang D (2013) Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. *Appl Energy* 104:801–809. <https://doi.org/10.1016/j.apenergy.2012.12.019>
58. Gandam PK, Chinta ML, Gandham AP, Pabbathi NPP et al (2022) A new insight into the composition and physical characteristics of corn-cob—substantiating its potential for tailored biorefinery objectives. *Fermentation* 8:12. <https://doi.org/10.3390/fermentation8120704>
59. Avanthi A, Banerjee R (2016) A strategic laccase mediated lignin degradation of lignocellulosic feedstocks for ethanol production. *Ind Crops Prod* 92:174–185. <https://doi.org/10.1016/j.indcrop.2016.08.009>
60. Kuila A, Mukhopadhyay M, Tuli DK, Banerjee R (2011) Accessibility of enzymatically delignified bambusa bambos for efficient hydrolysis at minimum cellulase loading: An optimization study. *Enzyme Res* 2011:8. <https://doi.org/10.4061/2011/805795>
61. Oliveira JAR, Komesu A, Martins LHS, Filho RM (2016) Evaluation of microstructure of açai seeds biomass untreated and treated with H₂SO₄ and NaOH by SEM RDX and FTIR. *Chem Eng Trans* 50:379–384. <https://doi.org/10.3303/CET1650064>
62. Md Salim R, Asik J, Sarjadi MS (2021) Chemical functional groups of extractives, cellulose and lignin extracted from native *Leucaena leucocephala* bark. *Wood Sci Technol* 55(2):295–313. <https://doi.org/10.1007/s00226-020-01258-2>
63. Wang H, Zhao L, Ren J, He B (2022) Structural changes of alkali lignin under ozone treatment and effect of ozone-oxidized alkali lignin on cellulose digestibility. *Processes* 10(3):559. <https://doi.org/10.3390/pr10030559>
64. Ditzel FI, Prestes E, Carvalho BM, Demiate IM, Pinheiro LA (2017) Nanocrystalline cellulose extracted from pine wood and corncob. *Carbohydr Polym* 157:1577–1585. <https://doi.org/10.1016/j.carbpol.2016.11.036>
65. Abderrahim B, Abderrahman E, Mohamed A, Fatima T, Abdes-selam T, Krim O (2015) Kinetic thermal degradation of cellulose, polybutylene succinate and a green composite: comparative study. *World J Environ Eng* 3:95–110. <https://doi.org/10.12691/WJEE-3-4-1>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.