Research Article

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Extrusion pre-treatment of cowpea (*Vigna unguiculata* (L.) Walp.) lignocellulosic sidestream to produce cellulose fibres

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Abstract

BACKGROUND: Various agricultural sidestreams have been demonstrated as feedstock to produce cellulose. To the best of our knowledge, there is no research work on the potential of agricultural sidestream from cowpea (*Vigna unguiculata* (L.) Walp.), a neglected and underutilised crop to produce cellulose fibres. Conventional methods to produce cellulose consume large amounts of chemicals (NaOH) and produce a high amount of effluent waste. Herein, we investigated extrusion pre-treatment without and with an alkali followed by bleaching as an alternative method to conventional alkaline pre-treatment followed by bleaching to produce cellulose fibres from cowpea sidestream.

RESULTS: Cellulose extracted by extrusion without and with mild alkali followed by bleaching consumed about 20 times less NaOH compared to the conventional method and produced less effluent waste. Extrusion with mild alkali followed by bleaching resulted in higher cellulose yield, purity, and crystallinity compared to extrusion without an alkali followed by bleaching. However, the conventional method resulted in higher cellulose yield, purity and crystallinity compared to extrusion pre-treatment followed by bleaching. Scanning electron microscopy revealed that micro-sized cellulose fibres with an average diameter of 10–15 μm were extracted using both methods. Notably, cellulose fibres extracted using extrusion pre-treatment were shorter than those extracted using the conventional method.

CONCLUSION: Extrusion pre-treatment is a promising continuous alternative to alkaline pre-treatment to produce micro-sized cellulose fibres from low-value, underutilised cowpea lignocellulosic sidestream, for potential use as a filler in composite plastics.
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Supporting information may be found in the online version of this article.

Keywords: cellulose; extraction; alkali; bleaching; lignocellulose; cowpea fibres

INTRODUCTION

Cellulose is one of the most abundant and renewable natural biopolymers. Cellulose and its derivatives are important in many different industries, such as packaging, biofuels and papermaking, due to its biocompatibility, biodegradability, abundance, and versatile mechanical, chemical and physical properties.[1-3] It can be produced from lignocellulosic biomass, one of the most abundant renewable resources, which is mainly composed of cellulose, hemicellulose and lignin.

The production of cellulose from lignocellulosic biomass involves successive chemical treatments. These include alkaline pre-treatment with 1–5% NaOH for up to 4 h to remove impurities and water-soluble hemicellulose, defibrillate the external cellulose microfibrils and depolymerise the native cellulose structure. This was followed by bleaching with 0.7–2% acidified sodium chlorite or H_2O_2 to remove lignin, thus yielding α-cellulose fibres.[4]

However, these conventional processes have several drawbacks, as they are time-consuming, use a large amount of chemicals and produce large amounts of effluents, and are therefore not environmentally friendly.[5]

This prompts a need to develop alternative greener technologies to produce cellulose. In recent years, mechanical treatments

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such as steam explosion,[4] ball milling[6] and extrusion[6,7] have been investigated as alternative methods for the valorisation of the lignocellulosic biomass. Extrusion, a conventional processing technology in polymer and food science, is a promising physical pre-treatment to produce cellulose. It offers several advantages, such as efficient mixing, rapid heat transfer, high solid loading and modification of processing conditions.[7,8] The high shear and temperature conditions during extrusion encourage the lignocellulosic biomass to undergo physical and chemical transformations.[9,10] In addition, effluent wastes are limited during extrusion as it does not produce liquid waste.[9]

Previously, agricultural sidestream, such as corn covers and corb,[11] rice husks,[12] banana pseudo stems, jute stems and pineapple leaf fibre,[13] have been used to produce cellulose and nanocellulose. However, the production of cellulose from the low-value agricultural sidestream of African crops, such as cowpea, is poorly documented. Cowpea is a legume that is grown mainly in sub-Saharan Africa for food and animal feed, and it is one of the most important indigenous African legumes.[14] It is a drought-tolerant crop that can grow with little agricultural input in arid and semi-arid regions and is suitable for intercropping with other cereals as it can fix nitrogen.[15] It is estimated that about 14.5 million hectares of land are utilised worldwide to produce about 6.5 million metric tons of cowpea.[16]

To the best of our knowledge, there is no notable reported research on the valorisation of low-value cowpea lignocellulosic sidestream to produce cellulose. This study seeks to demonstrate cowpea sidestream as a feedstock to produce cellulose and, moreover, to investigate extrusion pre-treatment without and with mild alkali as an alternative to conventional alkaline pre-treatment in the production of cellulose. The combination of high shear, temperature and pressure during extrusion will facilitate the penetration of superheated steam and chemicals (NaOH) into the lignocellulosic structure for defibrillation and separation of the components. Herein, we hypothesise that extrusion without and with mild alkali followed by bleaching will yield cellulose-rich fibres with properties comparable to cellulose fibres extracted by conventional alkaline pre-treatment followed by bleaching, for potential use as filler polymer composites.

EXPERIMENTAL

Materials
Cowpea sidestream was obtained from a farm (Farm Uitval, Vermass) located in North West province, South Africa, after grain harvesting in June 2021. Commercial cellulose (C6288) was procured from Sigma-Aldrich (Johannesburg, South Africa). All chemicals used were of analytical grade. The cowpea sidestream was washed with water, dried in an oven at 40 °C for 24 h, milled to <500 μm and sealed in containers until use.

Methods
Cellulose fibres were extracted from cowpea sidestream using (i) conventional alkaline pre-treatment followed by bleaching compared to (ii) extrusion without and with alkali followed by bleaching. 'Bleached' only refers to cowpea sidestream bleached without any pre-treatment. Commercial cellulose (C6288) was used as reference. Processing steps are summarised in Fig. 1.

Alkaline treatment
Alkaline treatment was conducted following a method described by Neto *et al.*,[17] with some modification. The cowpea sidestream

was treated with 2% NaOH solution (1:20 w/v fibre:liquor ratio, 400 g NaOH pellets per kg fibre) in a glass beaker for 2 h at 80 °C. The solids were filtered and washed with excess distilled water until neutral pH, followed by drying in a vacuum oven at 40 °C for 12 h.

Extrusion pre-treatment
The cowpea sidestream moisture content was adjusted to 50% by adding (i) distilled water (1:0.92 w/v fibre:liquor ratio) and (ii) a solution of 2% NaOH (1:0.92 w/v fibre:liquor ratio, 18 g NaOH pellets per kg fibre). The amount of liquor was significantly lower compared to alkaline pre-treatment. The cowpea sidestream were equilibrated at room temperature for 1 h before extrusion. The samples were extruded using a twin-screw extruder (Process 11, Thermo Scientific, Waltham, MA, USA) with an L/D of 40. The optimised extruder temperature profile was 80|100|100|120|120| 120|120|120 °C (feed zone to die), the feed rate was set at 0.15 kg h^{-1} and the screw speed was 220 rpm. The extruded samples were dry as they exited the extruder; they were washed with minimal distilled water to remove water-soluble components, dried in a vacuum oven 40 °C for 12 h and characterised.

Bleaching
The untreated, alkali-treated and the extruded (with and without alkali) samples were bleached using 1:1 (v/v) of 1.7 wt% sodium chlorite (NaClO$_2$) and acetate buffer (pH 4.71) at 80 °C for 4 h following the method described by Neto *et al.*[17] The solids were filtered and washed with distilled water to neutral pH, followed by drying in a vacuum oven at 40 °C for 12 h.

Analysis
The samples – untreated cowpea sidestream, alkali-treated followed by bleaching, and extruded (without and with alkali) followed by bleaching – were analysed. Commercial cellulose was used as a reference.

Cellulose, hemicellulose and lignin content. For the untreated cowpea sidestream the extractives were removed prior to characterization by Soxhlet extraction using 150 mL acetone for 4 h. The extractive-free untreated cowpea sidestream was used to determine cellulose, hemicellulose and lignin content.

Holocellulose. Theoretically, holocellulose is composed of cellulose and hemicellulose and was determined based on a method described by Carrier *et al.*,[18] with some modifications. About 150 mL of 0.2 mol L^{-1} glacial acetic acid (pH ∼2.78) was added to the Schott bottle containing 1 g samples and heated at 75 °C in a shaking water bath. Sodium chlorite (1 g) and acetic acid (0.2 mL) were added every hour for 4 h. The residues were cooled and washed with 1 L distilled water, followed by washing with 15 mL acetone. Finally, the residues were dried overnight in an oven at 103 °C, followed by weighing to determine holocellulose.

α-Cellulose. *α*-Cellulose is defined as the residue of holocellulose insoluble in 17.5% NaOH solution. About 100 mL of 17.5% NaOH was added to the Schott bottle containing holocellulose from the previous step and incubated in a shaking water bath at 30 °C for 30 min. The residue was washed with 1 L distilled water, followed by washing with 15 mL of 10% acetic acid to hydrolyse the degraded hemicellulose and cellulose. Next, the residue was washed with 500 mL hot distilled water followed by washing with 15 mL acetone. The remaining residue was dried overnight in an
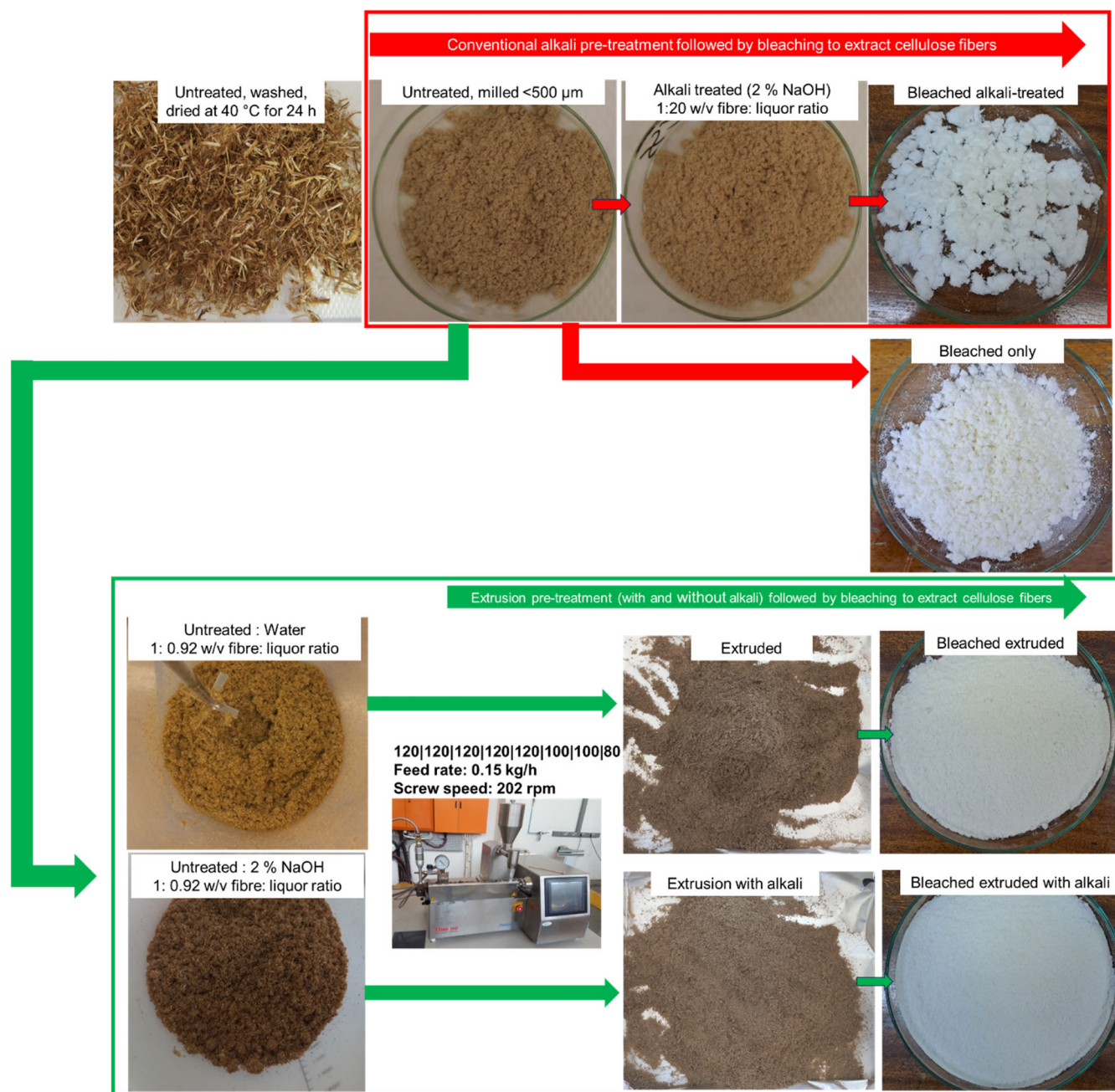
Figure 1. Processing steps showing conventional alkali pre-treatment followed by bleaching and extrusion pre-treatment (with and without NaOH) followed by bleaching to extract cellulose from cowpea lignocellulosic sidestream. The effect of bleaching only is also shown.

oven at 103 °C, followed by weighing to determine α-cellulose. Hemicellulose was calculated by subtracting α-cellulose from holocellulose. Samples were analysed in triplicate.

Klason lignin. Klason lignin is the insoluble residue after digestion with 72% sulfuric acid (H_2SO_4) and was determined according to the LAP-003 method for acid-insoluble lignin.[19] Samples were analysed in triplicate.

Fourier transform infrared (FTIR) spectroscopy

The structural changes during extraction were followed by FTIR spectroscopy (Spectrum 100 spectrometer, PerkinElmer, Waltham, MA, USA).[12,17] The samples were scanned at a resolution of 4 cm^{-1} using 32 scans and the spectra were collected at wavenumbers 4000 to 600 cm^{-1}.

X-ray diffraction (XRD)

The X-ray diffraction patterns of the samples were analysed using an XRD instrument (X'PERT PRO, PANalytical, Almelo, Netherlands). The instrument was operated at room temperature with an operating voltage of 45 kV and current of 40 mA. The instrument was operated in a continuous mode using a CuKα1 radiation source ($\lambda = 0.15406$ nm). The diffraction patterns were collected using Bragg-Brentano geometry in a scanning range of $2\theta = 5$–$40°$ with a scan step size of $0.02° \text{ s}^{-1}$.

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The crystallinity index (CrL) was determined from the diffraction patterns according to Segal *et al.*'s[20] method, using the formula below:

$$CrL\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad (1)$$

where I_{002} is the maximum intensity of the 002 diffraction peak, which has a diffraction angle at $2\theta = 22°$, and I_{am} is the lowest intensity at a diffraction angle around $2\theta = 18°$ and is scattered by the amorphous region of the sample.

Scanning electron microscopy (SEM)

The morphology of the samples was evaluated using SEM (JSM-7500, JOEL, Tokyo, Japan) at an acceleration voltage of 3.0 kV. Samples were coated with carbon prior to analysis.[17] Particle size analysis was conducted using ImageJ software to determine the diameter size distribution of cellulose. At least 40 particles were examined from two images of the same sample.

Thermogravimetric analysis (TGA)

The thermal stabilities of the samples during extraction were analysed using TGA Q5500 (TA Instruments, New Castle, DE, USA).[12] About 10 mg of samples was weighed in a platinum pan. The samples were heated from 25 to 600 °C at a heating rate of 10 °C min^{-1} under nitrogen gas (flow rate = 25 mL min^{-1}).

Statistical analysis

All experiments were repeated twice, unless otherwise stated. All data were analysed by multifactor analysis of variance using SPPSS version 20.0 statistical software for Windows (IBM Corp., Armonk, NY, USA). The significant difference between means was determined at $P < 0.05$ using Turkey's B test. The independent variables were the treatments, and the dependent variables were the measured values from the analysis.

RESULTS AND DISCUSSION

Cellulose, hemicellulose and lignin content

The chemical composition of all samples is shown in Table 1. To the best of our knowledge, there are no reports of the chemical composition of cowpea sidestream in the literature.

The untreated cowpea sidestream contained 393.1 g kg^{-1} cellulose, 275.0 g kg^{-1} hemicellulose and 136.4 g kg^{-1} lignin (Table 1), which is comparable to other lignocellulosic sidestream, such as corn stover, which contains 401–450 g kg^{-1} cellulose, 250–350 g kg^{-1} hemicellulose and 70–100 g kg^{-1} lignin,[11] and faba bean stems, which contain 390 g kg^{-1} cellulose, 320 g kg^{-1} hemicellulose and 160 g kg^{-1} lignin.[21]

Conventional alkaline pre-treatment reduced hemicellulose by 48%, resulting in higher α-cellulose content compared to the untreated sidestream (Table 1). Similarly, extrusion without and with an alkali reduced hemicellulose by 55% and 62%, respectively, resulting in higher α-cellulose content compared to the untreated sidestream (Table 1). Extrusion with an alkali resulted in higher α-cellulose content compared to extrusion without an alkali. Conventional alkali pre-treatment consumed higher amounts of NaOH (400 g NaOH kg^{-1} cowpea fibres), while extrusion with an alkali consumed 20 times less amount of NaOH (18 g NaOH kg^{-1} cowpea fibres). This demonstrates that extrusion pre-treatment without and with alkali as more environmentally friendly compared to the conventional alkali pre-treatment. It consumed fewer chemicals (NaOH), while removing hemicellulose and produced less chemical effluent waste compared to alkaline pre-treatment.

The screw configuration used in this study is shown in Fig. 2. The mixing elements (asterisk in Fig. 2) in zones 4–10 are areas of high shear and pressure in the extrusion barrel. The reverse elements (Fig. 2) may generate a backflow in the region between the mixing elements and the reverse element, creating an area of high pressure/compression. Therefore, it is possible that the combined effect of high shear mixing elements and superheated steam at high temperature (120 °C) causes areas of pressurisation and depressurisation in the extrusion barrel, leading to depolymerisation and solubilisation of non-cellulosic components, such as hemicellulose.

During extrusion without an alkali the combined effect of high shear, pressure and temperature may promote the penetration of superheated steam/water into the lignocellulosic fibres. The hot water/superheated steam can cleave the hemiacetal linkages in hemicellulose and facilitate the breakdown of ether linkages in the lignocellulosic biomass, thus solubilising hemicellulose.[22] Similarly, during extrusion with an alkali, the high shear, temperature and pressure may promote the penetration of the alkali into
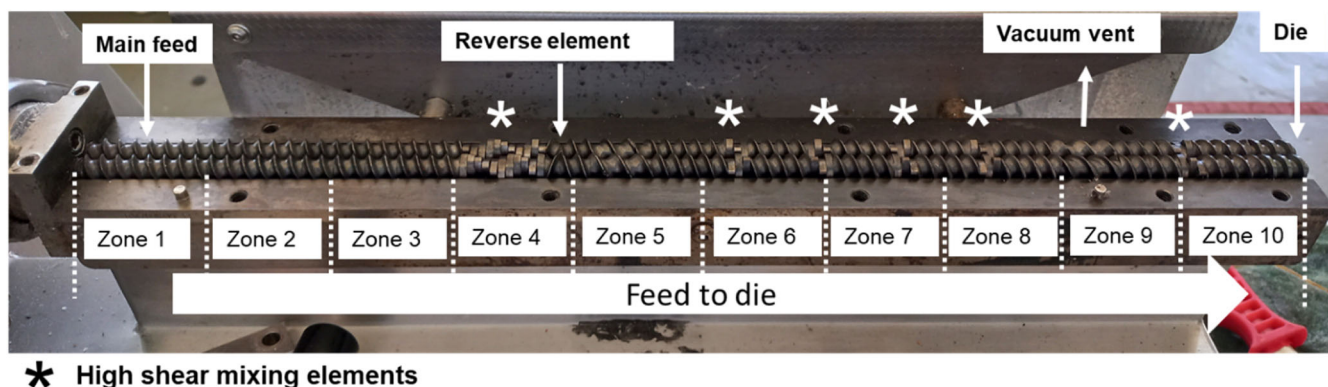
Table 1. Chemical composition of the untreated, extruded and chemically treated cowpea sidestream

Sample	Holocellulose (g kg^{-1})	α-cellulose (g kg^{-1})	Hemicellulose (g kg^{-1})	Lignin (g kg^{-1})	Solid yield[†] (%)	Cellulose yield[‡] (%)
Untreated	668.1 ± 6.1b	393.1 ± 6.0a	275.0 ± 12c	136.4 ± 2.0c	ND	ND
Bleached only	836.7 ± 12.7e	576.0 ± 9.0d	260.7 ± 21.0c	31.6 ± 0.6b	53%	57%
Alkali-treated	761.4 ± 2.6c	617.1 ± 13.4e	144.2 ± 14.4b	122.2 ± 19.2c	ND	ND
Bleached alkali-treated	912.6 ± 5.4f	895.1 ± 10.3h	17.5 ± 4.9a	0.0 ± 0.0a	37%	89%
Extruded	581.0 ± 26.0a	454.2 ± 10.8b	126.8 ± 19.1b	127.8 ± 4.8c	ND	ND
Bleached extruded	790.7 ± 8.8d	677.0 ± 3.6f	113.7 ± 5.8b	32.6 ± 1.2b	47%	66%
Extruded with alkali	589.2 ± 17.3a	482.4 ± 16.0c	106.9 ± 15.8b	131.9 ± 2.5c	ND	ND
Bleached extruded with alkali	833.3 ± 5.9e	721.7 ± 13.8g	111.6 ± 19.0b	32.3 ± 1.0b	49%	72%

Means with different letters in the same column are statistically significant. ND means not determined.
[†] Total solid yields after extraction relative to untreated cowpea sidestream.
[‡] Cellulose content of cellulose fibres in relation to cellulose content of untreated samples.

Figure 2. Screw configuration used during extrusion.

the lignocellulosic fibres. The alkali may cleave the acetyl groups in hemicellulose, resulting in its hydrolysis.[23] The addition of alkali during extrusion did not significantly ($P > 0.05$) affect the hemicellulose content compared to extrusion without an alkali. However, it resulted in higher α-cellulose content compared to extrusion without an alkali and untreated cowpea sidestream.

Bleaching alone removed lignin, resulting in higher amounts of α-cellulose, with hemicellulose unaffected compared to the untreated fibres. In addition, this treatment resulted in high solid yields but the lowest cellulose yield of 57%, compared to other treatments. The low cellulose yield compared to other treatments may be because bleaching was the only treatment that removed lignin without significantly affecting hemicellulose, in contrast to other treatments. Extrusion without an alkali, followed by bleaching, resulted in cellulose-rich fibres with α-cellulose content of 677 g kg^{-1} and cellulose yield of 66%. Meanwhile, extrusion with an alkali followed by bleaching resulted in even higher α-cellulose content of 721 g kg^{-1} and higher cellulose yield of 72% compared to extrusion without an alkali. Extrusion without and with an alkali, followed by bleaching, resulted in higher solid yields compare to the conventional method. However, conventional alkaline pre-treatment followed by bleaching resulted in purer cellulose fibres with higher α-cellulose content 895 g kg^{-1} and higher cellulose yields of 89%.

The removal of non-cellulosic components from lignocellulosic fibres improves the purity of cellulose. This can be followed by chemical changes, crystallinity and thermal stability determined by FTIR, XRD and TGA. These cellulose properties are important in its use as a filler and will be discussed in detail in the next sections.

Fourier transform infrared spectroscopy

FTIR spectroscopy was used to evaluate the structural changes during the extraction of cellulose from cowpea sidestream, and the results are shown in Fig. 3. The broad band around 3324 cm^{-1} corresponds to the free O—H stretching vibrations of the OH groups in cellulose.[24] The band between 2982 and 2830 cm^{-1} corresponds to the aliphatic saturated —CH stretching associated with the methylene groups in cellulose (Fig. 3).[25] The bands at 1159, 1024 and 894 cm^{-1} correspond to the C—O—C stretching vibration of the glycosidic ether band, the C—O—C pyranose ring stretching vibration and the C—H aromatic hydrogen, respectively.[26] These bands are associated with cellulose and the increase in their intensity after alkaline pre-treatment followed by bleaching, and after extrusion without and with alkali

followed by bleaching (Fig. 3), suggest the removal of non-cellulosic components to yield cellulose-rich fibres.

The band at 1737 cm^{-1} corresponds to the C=O stretching vibration of the acetyl and uronic ester groups in hemicellulose or the ester linkage of the carboxylic groups of p-coumaric and ferulic acids of lignin.[13] The disappearance of this band after alkaline pre-treatment followed by bleaching suggests the removal of hemicellulose and lignin (Fig. 3). Bleaching alone did not affect the intensity of this band compared to the untreated samples, suggesting that bleaching removed lignin without affecting hemicellulose. The intensity of this band reduced after extrusion without and with an alkali relative to the untreated sample (Fig. 3), due to the reduction of hemicellulose from 275 to 126 and 106 g kg^{-1}, respectively (Table 1). However, after bleaching of the extruded samples (with and without alkali) the intensity of this band increased due to the removal of lignin resulting in cellulose-rich fibres with hemicellulose as impurity.

The band at 1509 cm^{-1} corresponds to the —C=C— stretching vibration of aromatic rings of lignin.[24] This band disappeared after alkaline pre-treatment followed by bleaching (Fig. 3), suggesting the removal of lignin, as evidenced in Table 1. The intensity of this band decreased significantly after extrusion with and without alkali followed by bleaching and appeared as a very small band
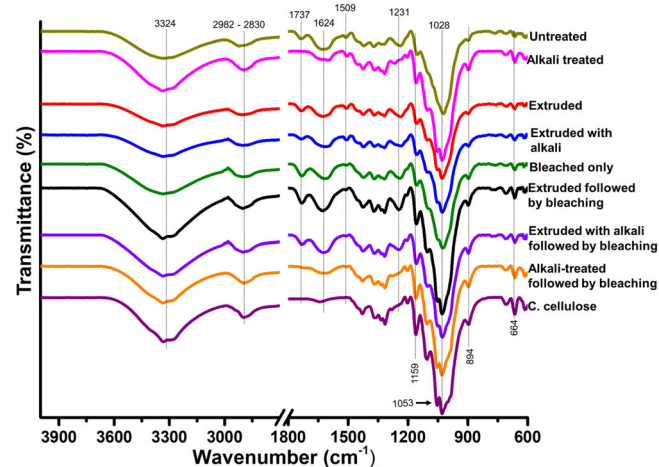

Figure 3. FTIR spectra of untreated, alkali-treated followed by bleaching, extruded (with and without alkali) followed by bleaching and commercial cellulose.

(Fig. 3), suggesting that not all lignin was removed (decreased from 136 to 32 g kg⁻¹) (Table 1).

In general, extrusion without and with an alkali followed by bleaching resulted in cellulose-rich fibres observed by an increase in the intensity of the bands corresponding to cellulose and a decrease in the intensity of the bands corresponding to non-cellulosic components. Cellulose fibres extracted using the conventional method displayed an FTIR spectrum that resembled commercial cellulose compared to the FTIR spectra of cellulose fibres obtained via extrusion pre-treatment followed by bleaching. This suggests that the conventional method resulted in purer cellulose fibres compared to the extrusion pre-treatment method.

X-ray diffraction

The XRD pattern of all samples is shown in Fig. 4. All X-ray diffractions displayed the main diffraction peaks at 22° (2θ), 15.4° (2θ) and 34° (2θ) (Fig. 4), which are assigned to cellulose type I.[27,28] The crystallinity index (CrL) of the samples are shown in Supporting Information, Table S1 and Fig. 4.

The untreated cowpea sidestream had the lowest crystallinity index of 55% (Fig. 4). Since cellulose is semi-crystalline, while hemicellulose and lignin are amorphous, the removal of non-cellulosic components would result in an increase in the index of crystallinity.[12,25] Therefore, the increase in crystallinity index to 77% after alkaline pre-treatment followed by bleaching suggested the removal of non-cellulosic components, resulting in cellulose-rich fibres with higher crystallinity index compared to untreated samples. Similarly, the increase in the crystallinity index after extrusion without and with alkali followed by bleaching to 72% and 75%, respectively (Fig. 4), suggested the removal of non-cellulosic components, resulting in cellulose-rich fibres. These results are consistent with chemical characterisation (Table 1) and FTIR (Fig. 3), which showed that extrusion followed by bleaching resulted in cellulose-rich fibres.

The conventional method resulted in cellulose fibres with a higher crystallinity index compared to the cellulose fibres extracted using extrusion pre-treatment (without and with alkali) followed by bleaching. This is because the conventional method resulted in cellulose fibres with higher semi-crystalline α-cellulose content (Table 1) compared to the extrusion method. In addition, the high temperature and high shear provided by the mixing

elements (Fig. 2) possibly disrupted the hydrogen bonds in cellulose, resulting in lower crystallinity index compared to cellulose extracted by conventional method. Commercial cellulose had a higher crystallinity index compared to the cellulose fibres extracted using both methods in this research due to greater purity of commercial cellulose.

Scanning electron microscopy

The effect of different extraction methods on the morphology of the resulting cellulose fibres was investigated by SEM and the images are shown in Fig. 5.

The untreated cowpea sidestream (Fig. 5a) displayed a compact structure mainly composed of cellulose, hemicellulose, lignin and some impurities. These non-cellulosic impurities act as cementing/binding materials around the cellulose fibres, giving a smooth outer layer.[29] After alkaline treatment, the cowpea sidestream (Fig. 5b) became rougher and fibre-like morphology began to develop – evidence of defibrillation of the compact lignocellulose structure. This is due to the removal of hemicellulose and other cementing/binding impurities, such as pectin and waxes as reported elsewhere.[24,29] The extruded cowpea sidestream without an alkali (Fig. 5f) and with an alkali (Fig. 5h) showed a fibre-like morphology, due to the removal of non-cellulosic components, such as hemicellulose and other impurities, as reported earlier. The combined effect of high shear, pressurisation and depressurisation in the extruder barrel, high temperature and removal of non-cellulosic components led to defibrillation, resulting in fibre-like morphology after extrusion (Fig. 5f,h). The high shear mixing elements (Fig. 2) led to physical breakdown of the fibres, resulting in shorter fibres in the extruded samples compared to the conventional alkaline pre-treatment.

The cowpea sidestream that was only bleached (Fig. 5d) revealed some limited defibrillation with a rougher morphology due to lignin removal (Table 1). Alkaline pre-treatment followed by bleaching (Fig. 5c) resulted in long individual micro-sized cellulose fibres (microfibrils) due to the removal of non-cellulosic components during successive chemical treatments. This was supported by chemical composition, FTIR and XRD. Extrusion without an alkali followed by bleaching (Fig. 5g) and extrusion with an alkali followed by bleaching (Fig. 5i) resulted in shorter micro-sized cellulose fibres due to the removal of non-cellulosic components. These fibres were shorter compared to those extracted by the conventional method, due to the high shear mixing elements (Fig. 2) that may cut the fibres, resulting in shorter cellulose fibres. The high shearing forces during mechanical treatments, such as ball milling, were also reported to reduce length and diameter of cellulose fibres.[29]

The particle size distributions of the cellulose fibres are shown in Supporting Information, Fig. S1. It can be noted that the cellulose fibres extracted with either method (conventional or extrusion) and commercial cellulose had a diameter size range between 5 and 30 μm, with the majority of the cellulose fibres having a diameter of 10–15 μm (Supporting Information, Fig. S1). This suggested that, regardless of the extraction method, the diameter of the resulting cellulose fibres was not severely affected. However, some differences in the shape and length were visually noted. Commercial cellulose (Fig. 5e) had rod-like short cellulose fibres, which were different from those extracted by either the conventional method or the extrusion method. This may be due to the difference in processing. For example, with commercial cellulose mild acid treatment might have been applied to α-cellulose to partially remove the amorphous parts, resulting in shorter fibres.[30]
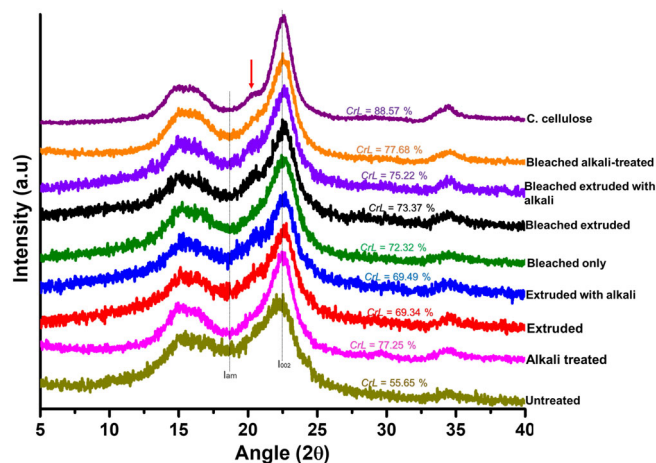

Figure 4. X-ray diffraction patterns of untreated, alkali-treated followed by bleaching, extruded (with and without alkali) followed by bleaching and commercial cellulose.
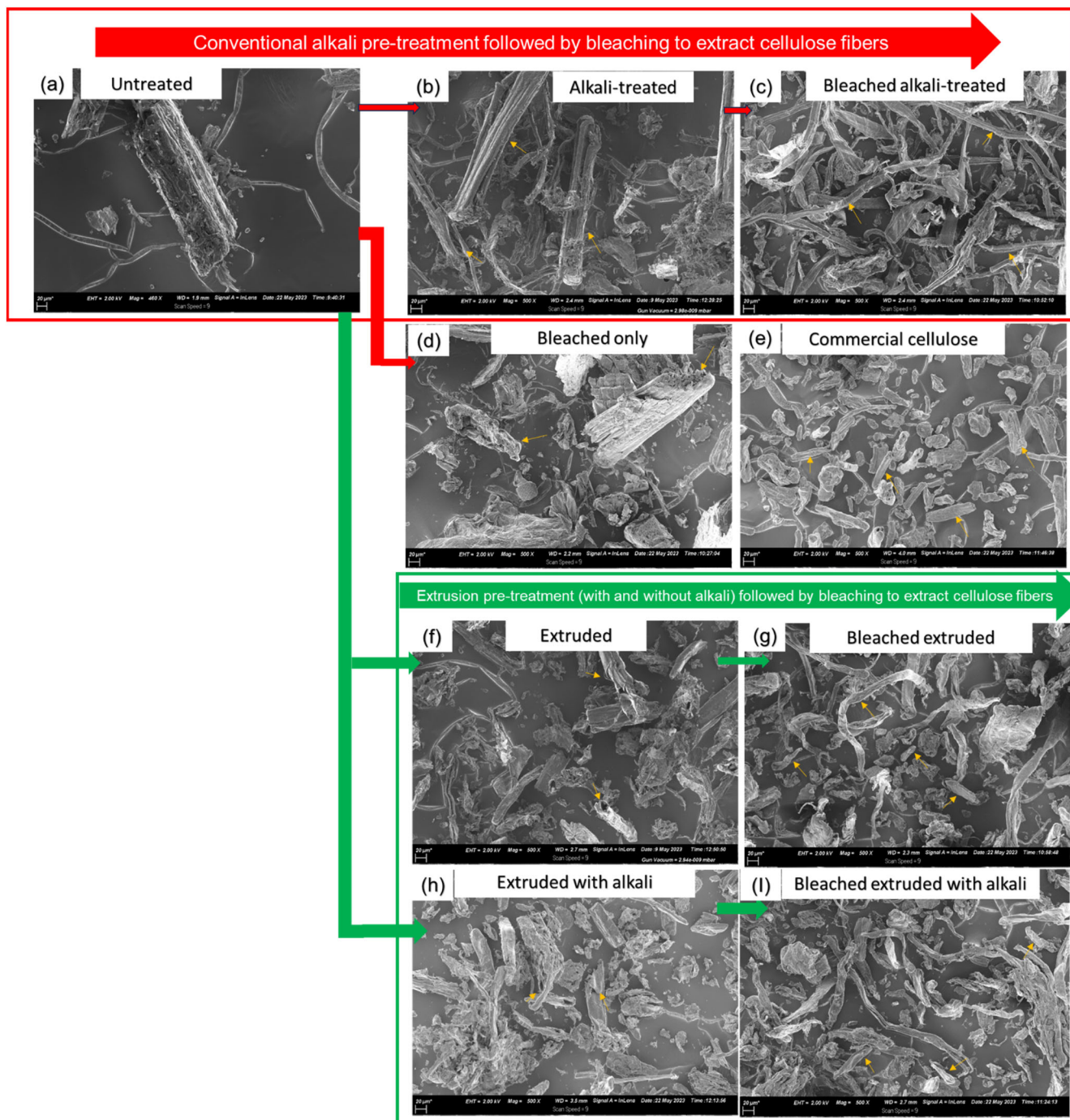
Figure 5. SEM images of untreated cowpea sidestream (a), alkali-treated (b) followed by bleaching (c), bleached only (d), commercial cellulose (e), extruded without alkali (f) followed by bleaching (g) and extruded with alkali (h) followed by bleaching (i). Scale bar 20 μm.

The cellulose fibres extracted by extrusion pre-treatment followed by bleaching were shorter than those obtained by the conventional method. Therefore, this raises the possibility of optimising extrusion conditions to produce shorter micro-sized cellulose fibres that resemble commercial microcrystalline cellulose, without further chemical treatment. Increasing the high shear mixing elements or multiple extrusion cycles of cowpea sidestream may result in shorter rod-like cellulose fibres. Previous research demonstrated that micro-sized cellulose can be reduced in size to nano-fibrillated cellulose by multiple extrusion cycles.[31] However,

the extrusion conditions should be optimized in order not to degrade the cellulose structure during multiple extrusion cycles or with more mixing elements.

Thermogravimetric analysis
TGA and derivative TGA (dTGA) (Fig. 6) show the effect of extraction method on the thermal stabilities of the resulting cellulose fibres. The detailed values from TGA and dTGA are shown in Supporting Information, Table S2.
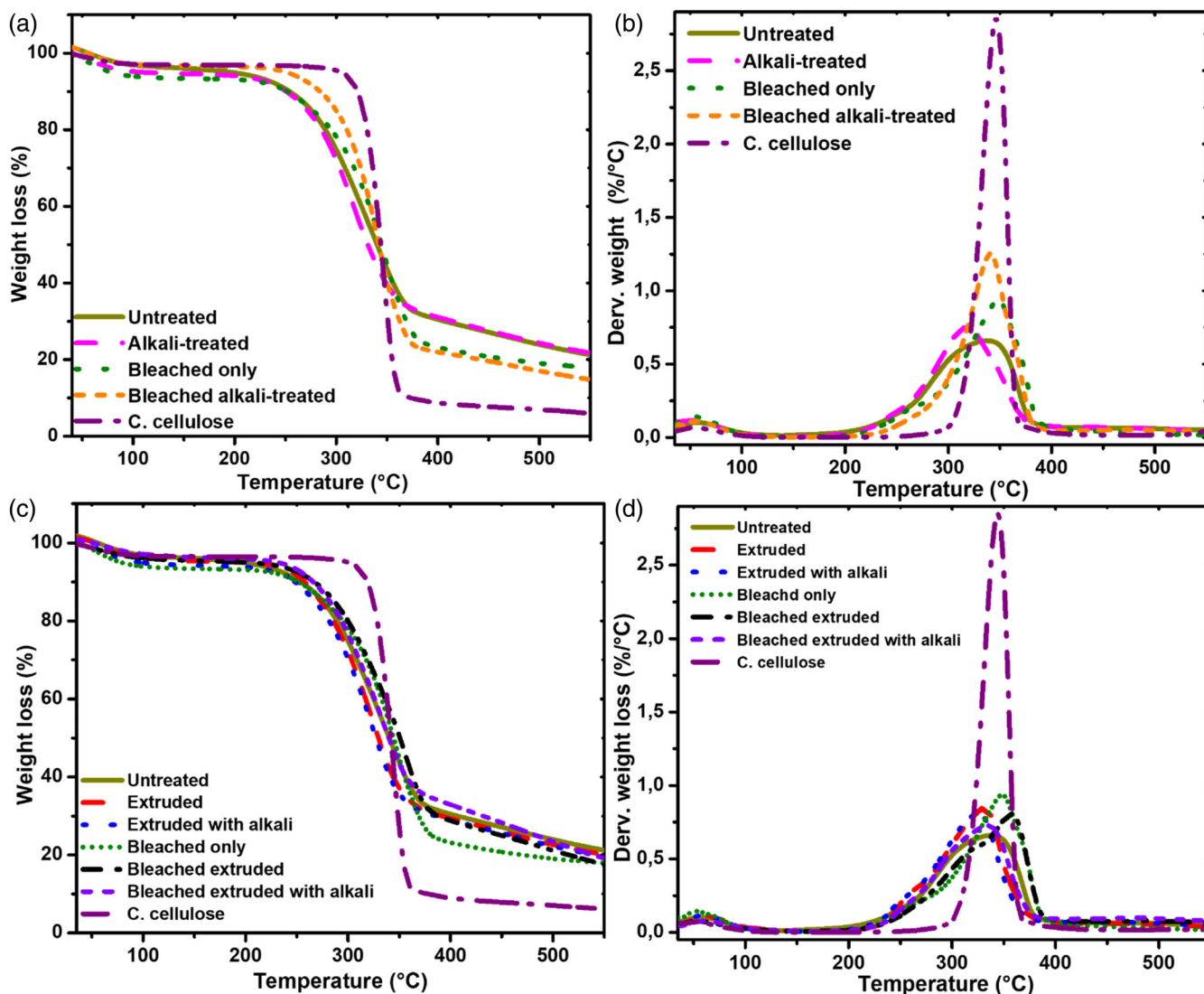
Figure 6. Weight loss *versus* temperature of (a) chemically treated, (c) extruded cowpea sidestream compared to commercial cellulose and derivative weight loss *versus* temperature of (b) chemically treated, (d) extruded cowpea sidestream compared to commercial cellulose.

Weight loss below 100 °C in all the samples corresponds to the evaporation of moisture or low-molecular-weight compounds (Fig. 6a,c). The untreated cowpea sidestream displayed a broad degradation step above 300 °C, which narrowed after alkaline pre-treatment followed by bleaching (Fig. 6b). Similarly, extrusion without and with an alkali followed by bleaching resulted in the narrowing of the main degradation step (Fig. 6d). Hemicellulose degradation occurs around 220–315 °C, while cellulose degradation occurs between 300 and 400 °C. Lignin is a complex molecule whose degradation occurs over a broad temperature range between 150 and 900 °C.[32] Therefore, the narrowing of the main degradation step after alkaline pre-treatment or extrusion without and with alkali followed by bleaching suggests the degradation of purer cellulose, due to the removal of non-cellulosic components demonstrated by chemical composition, FTIR and XRD. However, the cellulose fibres extracted by extrusion without and with alkali followed by bleaching displayed a small shoulder around 250 °C (Fig. 6d) – evidence of incomplete hemicellulose removal, as discussed earlier (Table 1).

The untreated cowpea sidestream had initial degradation temperature (T_o) and maximum degradation temperature (T_{max}) of 263.90 and 338.5 °C, respectively (Supporting Information, Table S2). T_o and T_{max} decreased after alkaline pre-treatment, indicating lower thermal stability relative to the untreated samples (Supporting Information, Table S2). During alkaline treatment (mercerisation), the hydrogen in —OH groups of cellulose may be substituted by the Na^+ ion to form —O^-Na^+,[33] thus weakening the hydrogen bonds in cellulose to decrease thermal stability. Similarly, extrusion without an alkali decreased the thermal stability relative to the untreated samples, which decreased further after extrusion with an alkali (Fig. 6(c) and Supporting Information, Table S2). During extrusion, the high shearing forces and high temperature may cause physical breakdown of cellulose fibres to decrease the thermal stability compared to untreated samples. With the addition an alkali during extrusion, the combined effect of shear, temperature and mercerisation may further decrease the thermal stability compared to untreated sample extruded without an alkali.

Bleaching alone increased the thermal stability, as indicated by higher T_{50} and T_{max} compared to the untreated cowpea side-stream (Supporting Information, Table S2). This was due to lignin removal, as indicated earlier. Alkaline pre-treatment followed by bleaching led to a significant increase in thermal stability compared to untreated samples (Fig. 6a and Supporting Information, Table S2). This is due to the higher purity and crystallinity of the resulting cellulose fibres. Extrusion without and with alkali followed by bleaching also resulted in higher thermal stability compared to the unbleached counterparts (Fig. 6c,d). This was due to the removal of lignin during bleaching to yield higher cellulose purity and crystallinity. However, extrusion pre-treatment with an alkali followed by bleaching resulted in cellulose fibres with lower thermal stability compared to extrusion without an alkali followed by bleaching due to mercerisation of cellulose during extrusion with an alkali.

The cellulose fibres extracted by either conventional method or extrusion without and with alkali followed by bleaching had lower thermal stability compared to commercial cellulose. However, their initial degradation temperature was above 260 °C, making them suitable for use as a filler in composite plastics, as most polymers are processed below 250 °C.

CONCLUSIONS

Low-value cowpea lignocellulosic sidestream can be used as a feedstock to extract cellulose fibres. Extrusion with an alkali followed by bleaching resulted in cellulose-rich fibres with higher yield, purity and crystallinity compared to extrusion without an alkali followed by bleaching. However, conventional alkaline pre-treatment followed by bleaching resulted in cellulose fibres with higher yield, purity and crystallinity compared to extrusion pre-treatment followed by bleaching. The extrusion pre-treatment method consumed 20 times less NaOH compared to conventional alkaline pre-treatment method and produced less effluent waste, making it relatively environmentally friendly. In conclusion, extrusion pre-treatment is a promising continuous alternative to the conventional method in the production of micro-sized cellulose fibres from the low-value and underutilised cowpea sidestream, for potential use as a filler in composite plastics.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Mondli Abednicko Masanabo: conceptualisation, methodology, formal analysis, investigation, software, writing – original draft, writing – review and editing. Janne Tapani Keränen: conceptualisation, validation, writing – review and editing, supervision. Suprakas Sinha Ray: conceptualisation, validation, funding acquisition, resources, supervision, writing – review and editing. M Naushad Emmambux: conceptualisation, validation, funding acquisition, resources, supervision, writing – review and editing, project administration. All authors have read and agreed to the published version of the manuscript.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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