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Sugarcane waste as a valuable source of lipophilic molecules

Thomas M. Attard[a], C.Rob McElroy[a], Camila A. Rezende[b], Igor Polikarpov[c], James H. Clark[a], Andrew J. Hunt[a,*]

[a] Department of Chemistry, University of York, Heslington, York YO10 5DD UK
[b] Universidade Estadual de Campinas (UNICAMP), Instituto de Química, PO Box 6154, Campinas, SP 13083-970, Brazil
[c] Grupo de Biotecnologia Molecular, Instituto de Física de São Carlos, Universidade de São Paulo Av., Trabalhador Sãocarlense, São Carlos, SP 400 13566-590, Brazil

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ABSTRACT

Extraction of high-value products from agro-industrial waste is an important component for the development of a sustainable bioeconomy. In this work, natural wax extraction was carried out on different types of sugarcane waste (rind, leaf and bagasse) using supercritical CO_2 ($scCO_2$). Substantial quantities of long-chain aldehydes and *n*-policosanols (nutraceutical compounds) were found in the rind (83% of total composition). Interestingly, the wax obtained from the leaf residues varied from other types of waxes from sugarcane waste, with low aldehyde and *n*-policosanol contents (normally found in high quantities) and considerable amounts of high-value triterpenoids (169 ±6 mg/g wax), which have well-known medicinal properties. The use of sugarcane leaf residues for the extraction of waxes has not been previously considered, though the amount of these residues increased significantly after the switch to green harvesting. Sugarcane bagasse wax showed the highest ester composition (37 ± 1.5 mg/g of wax), which can be useful in a host of applications, ranging from cosmetics to hard wax polishes, lubricants, coatings and plasticisers.

1. Introduction

Plant waxes refer to the complex mixture of surface lipids that covers the aerial tissues of herbaceous plants (Kunst and Samuels, 2003). These hydrophobic compounds have found uses in a host of applications from cosmetics to nutraceuticals, coatings, polishes, detergents among other things (Sin et al., 2014). Natural waxes are in high demand due to the diminishing supply of petroleum waxes, together with the transition to greener products by consumers. Currently, natural waxes contribute to only 4% of the total global wax production. Waxes from plant wastes are preferable, so as not to compete with the agricultural industry and with food production (Kline, 2011).

Waste from sugarcane crops (*Saccharum officinarum* L.) can be one possible source of natural waxes. Sugarcane is a C_4 plant, which has experienced nearly a six fold increase in the global harvest from 1950 to 2007 (global total production of 1.5 billion tons) (Zuurbier and van de Vooren, 2008). The harvesting and processing of sugarcane produces a significant amount of residues including waste leaves, rind and bagasse. While the leaves are disposed of during sugarcane harvesting, the rind (the hard outer layer of the plant stalk) and the bagasse waste are obtained during sugarcane milling for sugar or ethanol production. Recently, traditional manual harvesting methods which require pre-burning of the sugarcane fields have come under environmental pressure and resulted in an increase in mechanized green harvesting (Eggleston et al., 2014). This has resulted in an appreciable increase in the quantities of sugarcane leaf residues produced (Eggleston et al., 2014). Sugarcane bagasse, in turn, refers to the lignocellulosic residue, remaining from conventional milling of sugarcane and is collected in large amounts after sugarcane processing (Pandey et al., 2000; Zuurbier and van de Vooren, 2008). In a typical sugar mill, the processing of one metric ton of sugarcane gives rise to around 270 kg of bagasse (with 50% moisture), which equates to approximately 135 kg of dry matter (Baudel et al., 2005; Pimenta and Frollini, 1997).

Sugar and ethanol plants normally use around 50% of the residual bagasse to generate heat and power. The rest is normally stockpiled on site, posing an environmental problem to both the mills and the surrounding districts, since long-period storage could increase the risk of spontaneous combustion (Lavarack et al., 2000). There have been various reports highlighting the use of sugarcane bagasse in a variety of applications, ranging from animal feed to the

* Corresponding author.
E-mail addresses: carezende@gmail.com (C.A. Rezende), ipolikarpov@ifsc.usp.br (I. Polikarpov), andrew.hunt@york.ac.uk (A.J. Hunt).

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production of various industrial enzymes (cellulases, lipases etc.), chemicals, pulp and paper (Benjamin et al., 2013; Lavarack et al., 2000; Rocha et al., 2012).

Wax extraction from biomass is normally carried out using conventional solvents such as dichloromethane (DCM), chloroform, hexane and toluene; which have become restricted due to various toxicological and environmental problems (Deswarte et al., 2006). An excellent alternative solvent for the extraction of natural products is supercritical carbon dioxide ($scCO_2$), since carbon dioxide is non-flammable, non-toxic and widely available (Subramaniam et al., 1997). Furthermore, it is a recyclable and non-regulated solvent (Subramaniam et al., 1997).

Herein, the supercritical extraction of waxes from the sugarcane leaves, rind and bagasse was investigated. The hydrophobic molecules present in each wax were characterized, quantified and their potential applications were identified in this work, since these molecules are added-value products. The waxes from each sugarcane waste were compared and contrasted in order to determine suitable applications for these products.

2. Material and methods

2.1. Sugarcane material

One-year old sugarcane plants were provided by Fazenda Cercadinho (Casa Branca, SP, Brazil). The leaves and rind were separated from the stalk. The rind, formed by a thin, darker and harder external layer of the plant stalk, was removed to leave the internal part of the stalk. The latter part was milled to remove the majority of the sugar juice; the solid waste produced is the bagasse. All samples were rinsed to remove surface dust (leaf and rind), or residual sugar (bagasse). They were then dried in a convection oven at 60 °C for 24 h. The samples were ground prior to supercritical extraction and passed through a 2 mm sieve.

2.2. Supercritical fluid extraction (SFE)

The $scCO_2$ extractions were carried out using a SFE-500 provided by Thar technologies. Supercritical fluid grade carbon dioxide (99.99%) was used to carry out the extractions. A total of 100 g of milled sugarcane residues (rind, leaves or bagasse) was placed into the 500 cm^3 extraction vessel and connected to the extraction system. The required temperature and pressure for the extraction were applied. The reaction vessel was heated to 50 °C and kept at this temperature for 5 min to reach thermal equilibrium. An internal pump was used to obtain the required pressure (350 bar). The system was run in dynamic mode, in which the carbon dioxide which contained the epicuticular lipids, was allowed to flow into the collection vessel. A flow rate of 40 g min^{-1} of liquid CO_2 was applied and the extraction was carried out for 4 h. When the extraction was terminated, the system was depressurized over a period of 4 h. The wax was collected by rinsing the collection vessel twice with approximately 100 ml of dichloromethane (DCM). The solvent was removed in vacuo. The crude wax product was weighed and the extraction yield (%) was calculated. The plant material was then removed and the extraction vessel cleaned.

2.3. Soxhlet extraction of biomass

11 g of milled biomass (sugarcane rind or leaves) was placed in a Soxhlet thimble which was inserted into the Soxhlet apparatus. This was fitted to a 250 ml round bottom flask containing hexane (200 ml). A Radleys Discovery Technologies 2006T thermocouple was used to monitor the temperature during the extraction. The solution was allowed to reflux for 4 h. The resulting solution was filtered (to remove any biomass present in the product) and the solvent was removed in vacuo. The samples were further dried at room temperature for 24 h before weighing to ensure the removal of traces of residual solvent. The crude wax product was weighed and the % yield calculated. Three extractions were carried out and an average % yield calculated.

2.4. Derivatisation prior to high temperature-gas chromatography (HT-GC)

A total of 30 mg of crude wax extract was silylated by adding 200 μl N,O-*bis*-(trimethylsilyl)-trifluoro-acetamide and 100 μl toluene. The closed vial was heated in an oven for 30 mins at 75 °C.

2.5. High temperature-gas chromatography (HT-GC) procedure

HT-GC analysis was performed using an Agilent Technologies 6890N Network GC System and a ZB-5HT capillary column (30m × 250 μm × 0.25 μm nominal). A 22.35 psi constant pressure was applied and helium was used as the carrier gas. The injector temperature and the flame ionisation detector temperature were maintained at 300 °C. The samples were injected by automated injection (1 μl injection volume) with a split ratio of 5:1. An initial oven temperature of 60 °C was maintained for 1 min, then the temperature was increased at a ramp rate of 8 °C min^{-1} until 360 °C.

Quantification of the lipid components was carried out by means of internal standard calibration and response factors (R_f). Six point linear calibration graphs were produced using external standards for the quantification of hydrophobic compounds.

2.6. High temperature-gas chromatography mass spectrometry (HT-GC–MS)

HT-GC–MS was performed on a Perkin Elmer Clarus 500 GC, coupled with a Clarus 500 quadrupole mass spectrometer, with a DB5HT capillary column (30m × 250 μm × 0.25 μm nominal) at constant pressure of 22.35 psi. The carrier gas used was helium. The temperature of the injector was 360 °C and the flow rate was set to 1.00 ml min^{-1}. The initial oven temperature was kept at 60 °C for 1 min, and then it was ramped at a rate of 8 °C min^{-1} until 360 °C and held for 30 min. The Clarus 500 quadrupole mass spectra was operated in the electron ionisation mode (EI) at 70 eV, a source temperature of 300 °C, quadrupole at in the scan range of 30–1200 amu per second.

Another method was developed for the analysis of wax esters. The temperature of the injector was 380 °C and the flow rate was set to 1 ml min^{-1}. The initial oven temperature was kept at 100 °C for 1 min. The temperature was then ramped at a rate of 10 °C min^{-1} until 380 °C and held for 20 mins. The Clarus 500 quadrupole mass spectra was operated in the electron ionisation mode (EI) at 70 eV, a source temperature of 300 °C, and a quadrupole in the scan range of 30–1200 amu per second.

The data was collected with the PerkinElmer enhanced TurboMass (Ver5.4.2) chemical software and the compounds were identified by comparison of mass fragmentation patterns with spectra contained in the NIST library (v. 2.2) and by direct comparison with standard compounds.

2.7. Differential scanning calorimetry (DSC)

The thermal characteristics of the wax samples were measured on a Q2000 modulated differential scanning calorimeter. The wax extract was weighed (5.3 mg) into a closed aluminum pan and analyzed under nitrogen using a three-stage heating profile: 1. First heating from 20 to 105 °C, to remove the sample thermal history, followed by an isotherm (at 105 °C) for 1 min; 2. Cooling to −10 °C,

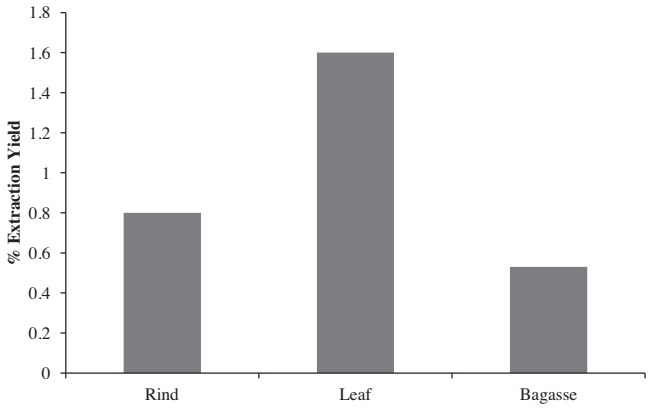

Fig. 1. Extraction yields (%) of wax from sugarcane rind, leaves and bagasse.

followed by a second isotherm for 1 min; and 3.Second heating from −10 to 105 °C, followed by a 1 min isotherm (at 105 °C). All the heating and cooling rates were 10 °C min^{-1}. The melting point range was determined using the differential scanning calorimetry (DSC) curves of the second heating cycle.

3. Results and discussion

The yields of lipophilic extractives from the rind, leaves and bagasse are summarized in Fig. 1. The highest yield of wax was obtained from the leaves, which accounted for 1.60% of the dry biomass, followed by the rind (0.8%) and the stem (0.53%).

GC and GC–MS analyses were used to characterise the underivatized and silylated extracts using a high temperature capillary column and methods to allow the elution and determination of high-molecular weight compounds such as sterols, triterpenoids and wax esters.

Table 1 indicates the identities and quantities of the different lipophilic constituents of the waxes extracted from the rind, leaves and bagasse of sugarcane. The visual appearance and texture of the waxes (Supplementary- Fig. S1) differed for each type of sugarcane waste. GC and GC–MS analyses indicate that there is a significant difference in both the type and quantity of hydrophobic compounds found within the waxes from the rind, leaves and bagasse. These are summarized in Fig. 2.

The wax from sugarcane rind differs from other plant waxes because it contains substantial quantities of long-chain fatty aldehydes and long-chain policosanols, which comprised 56% and 27% of the wax composition, respectively. Octacosanal (375.2 ± 86.9 mg/g of wax) and 1-octacosanol (237.9 ± 30.3 mg/g of wax) were the predominant chain length for each group (Fig. 3A and B).

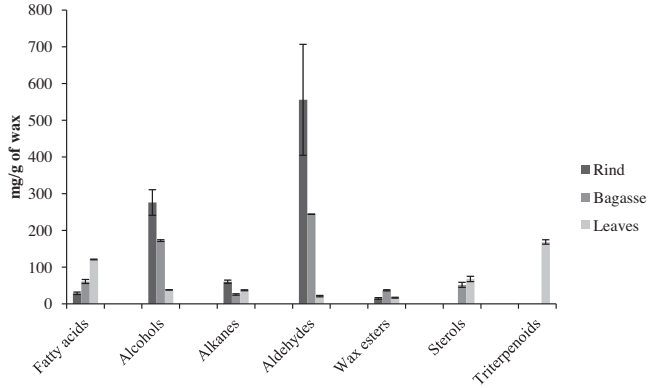

Fig. 2. Type and distribution of hydrophobic compounds in the extractives from sugarcane rind, leaves and bagasse, quantified by GC.

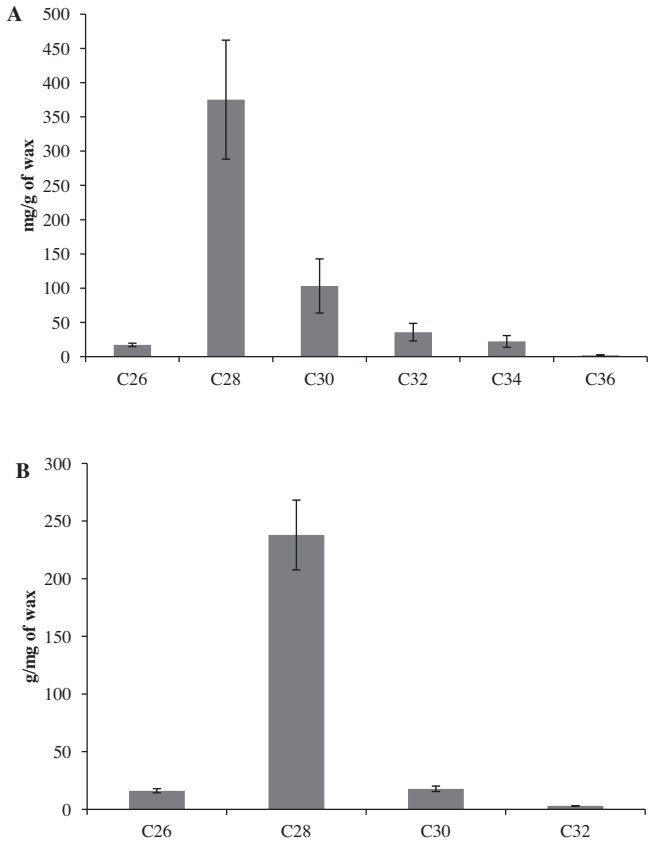

Fig. 3. (A Fatty aldehyde distribution 3B) Fatty alcohol distribution from the rind extractives, quantified by GC.

Long-chain fatty aldehydes, such as octacosanal, have been found to have health benefits, as effective agents for preventing and treating osteoporosis (Chinen, 2006). It has been shown that these long-chain fatty aldehydes can be safely and effectively administered in food and drink (Chinen, 2006). *n*-Policosanols are highly sought after as they have a wide variety of potential applications, most notably in the prevention and treatment of a variety of cardiovascular-related conditions such as poor arterial function, hypercholesterolemia, poor antioxidant status and intermittent claudication (Marinangeli et al., 2010). They are also shown to lower cholesterol levels (Varady et al., 2003). Furthermore, apart from medicinal uses, policosanols may also have potential cosmetic applications, such as in anti-acne formulations (Majeed et al., 2007). It would be relatively straightforward to isolate and purify these compounds from the extract since they make up the majority of the wax in terms of composition. Apart from these compounds, relatively small quantities of long-chain alkanes, long-chain fatty acids and wax esters were also present.

The results obtained from the crude extract of sugarcane rind are comparable with literature (Asikin et al., 2012; Lamberton and Redcliffe, 1960; Nuissier et al., 2002; Purcell et al., 2005; Rutherford and van Staden, 1996). However, a much wider variety of compounds were found in the wax obtained from the leaves when compared to the rind. Fig. 4 shows GC–MS chromatograms of unsilylated waxes from the sugarcane leaves and rind, where a stark contrast may be observed when comparing the extractives. A greater variety of families of compounds are found in the leaf wax including *n*-alkanes, saturated and unsaturated fatty acids, fatty-alcohols, fatty aldehydes, wax esters, sterols, triterpenoids

Table 1
Quantification of compounds found in lipophilic extractives from rind, leaves and bagasse of sugarcane (in μg/g of plant), quantified by GC.

Compounds	Rind (μg/g of dry plant)	Leaves (μg/g of dry plant)	Bagasse (μg/g of dry plant)
Hexanoic acid	17.2 ± 1.6	1.4 ± 0.1	3.3 ± 0.04
Heptanoic acid	–	1.3 ± 0.05	0.3 ± 0.03
Octanoic acid	25.1 ± 1	2.8 ± 0.1	1.2 ± 0.05
Nonanoic acid	–	2.7 ± 0.2	1.6 ± 0.03
Decanoic acid	0.8 ± 0.1	7.7	0.5
Dodecanioc acid	4.5 ± 0.3	112.4 ± 2.4	3.1
Tetradecanioc acid	3.5 ± 0.4	104.5 ± 0.3	3 ± 0.04
Pentadecanioc acid	2.2 ± 0.2	13.1	1.9 ± 0.03
Hexadecanoic acid	126.6 ± 15.3	866.8 ± 4.2	99.9 ± 1.1
Heptadecanoic acid	–	29.5 ± 0.1	1.8 ± 0.04
Octadecanoic acid	15.9 ± 1.7	94.1 ± 0.7	13.8 ± 1.4
Nonanoic acid	–	9.1 ± 0.3	0.4
Eicosanoic acid	14 ± 1.2	119.9 ± 1.1	18.1 ± 0.3
Heneicosanoic acid	–	8.2 ± 0.2	0.7 ± 0.1
Docosanoic acid	TR	48.7 ± 0.9	6.9 ± 0.1
Tricosanoic acid	–	17.5	4.3 ± 0.1
Tetracosanoic acid	3.1 ± 2.4	75.3 ± 1.1	6.2 ± 0.1
Pentacosanoic acid	–	–	10.5 ± 4.2
Hexacosanoic acid	–	TR	TR
Octacosanoic acid	–	TR	65.1 ± 2.4
Tricontanoic acid	–	TR	33.7 ± 0.3
Total saturated fatty acids	212.9 ± 24.2	1515 ± 11.7	277.3 ± 10.4
9-Octadecenoic acid	3.4	8.2 ± 0.2	1.3 ± 0.1
9,12-Octadecadienoic acid	3.2 ± 0.4	215.8 ± 2.1	19.9 ± 10.2
9,12,15-Octadecatrienoic acid	1.5 ± 0.2	197.2 ± 2.2	21.3 ± 8.5
Total unsaturated fatty acids	8.1 ± 0.6	421.2 ± 4.5	42.5 ± 18.8
Octanedioic acid	2.2 ± 0.4	–	–
Nonanedioic acid	8.2 ± 1.5	11.9 ± 0.8	3.7 ± 0.1
Decanedioic acid	0.7 ± 0.1	–	–
Total saturated di-fatty acids	11.1 ± 2	11.9 ± 0.8	3.7 ± 0.1
Tetracosanol	4.5 ± 0.4	–	3.1 ± 0.1
Hexacosanol	129.1 ± 14.5	19.6 ± 1.5	43.3 ± 0.2
Octacosanol	1902.8 ± 242.3	110 ± 3.3	716.7 ± 4.7
Triacontanol	142.7 ± 19	90.9 ± 1.1	91.5 ± 1.4
Dotriacontanol	23.3 ± 1.7	393.2 ± 2.1	50.5 ± 1.1
Tetratriacontanol	5.8 ± 0.6	–	8.5 ± 2.4
Total saturated fatty alcohols	2208.2 ± 278.5	613.7 ± 8	913.6 ± 9.9
Tetracosanal	–	23.6 ± 1.3	–
Hexacosanal	137.5 ± 19.8	35.2 ± 4.5	36.6 ± 0.4
Octacosanal	3001.5 ± 694.8	81.3 ± 11.1	807.1 ± 5.8
Triacontanal	825.6 ± 315.9	199.3 ± 14.1	260.3 ± 2
Dotriacontanal	286.7 ± 102.2	TR	111.7 ± 1.4
Tetratriacontanal	177.3 ± 68.8	–	78.8 ± 0.1
Hexatriacontanal	16.4 ± 6.1	–	–
Total saturated fatty aldehydes	4445 ± 1207.6	339.4 ± 31	1294.5 ± 9.7
Tricosane	3.6 ± 0.3	8.2 ± 0.1	1
Pentacosane	7.4 ± 0.2	5 ± 2.7	4.1
Heptacosane	113.1 ± 3.3	20.2 ± 0.1	41.1 ± 0.3
Octacosane	2 ± 0.7	–	1.8 ± 0.03
Nonacosane	41.4 ± 8.2	45.5 ± 0.5	22.5 ± 0.3
Hentriacontane	315.8 ± 23.3	167.2 ± 2.4	33.6 ± 1.7
Triatriacontane	–	303.6 ± 13	32.4 ± 0.5
Pentatriacontane	–	44.6 ± 1.9	–
Total hydrocarbons	483.3 ± 36	594.3 ± 20.7	136.5 ± 2.8
Campesterol	–	–	80.2 ± 7.3
Stigmasterol	–	464.4 ± 0.8	79.9 ± 23.3
β-Sitosterol	–	623.4 ± 114.9	115 ± 6.1
Total sterols	–	1087.8 ± 115.7	275.1 ± 36.7
Crusgallin	–	748.6 ± 23.1	–
Cylindrin	–	209.1 ± 31.1	–
Arundoin	–	712.4 ± 3.2	–
Simiarenol	–	533.7 ± 23.7	–
Friedelin	–	495 ± 14.1	–
Total Triterpenoids	–	2698.8 ± 95.2	–
Wax ester C_{38}	–	20.6 ± 0.7	1.2
Wax ester C_{40}	1.4 ± 0.2	12.6 ± 2	2.8
Wax ester C_{42}	6.9 ± 1.2	15.1 ± 1.6	8.4 ± 0.3
Wax ester C_{43}	1.7 ± 0.3	8.2 ± 0.4	3.1
Wax ester C_{44}	37.2 ± 2.9	41.1 ± 0.3	46.7 ± 0.6
Wax ester C_{45}	1.6 ± 0.1	14.7 ± 1.8	6 ± 0.2
Wax ester C_{46}	14 ± 2.1	37.8 ± 1.9	28 ± 0.2

Table 1 (*Continued*)

Compounds	Rind (μg/g of dry plant)	Leaves (μg/g of dry plant)	Bagasse (μg/g of dry plant)
Wax ester C_{47}	1.4 ± 0.1	2.8 ± 0.2	3.4 ± 0.2
Wax ester C_{48}	11 ± 1.1	54.2 ± 3.9	26.6 ± 0.4
Wax ester C_{49}	0.8 ± 0.2	4.3 ± 0.4	3.5 ± 0.1
Wax ester C_{50}	6.2 ± 0.1	29 ± 4.2	14.5 ± 0.2
Wax ester C_{51}	1.1 ± 0.1	–	3.7 ± 0.1
Wax ester C_{52}	3.7 ± 0.4	20.6 ± 0.7	11.1 ± 0.1
Wax ester C_{53}	1.3 ± 0.1	–	3.3 ± 0.1
Wax ester C_{54}	3.4 ± 0.6	11 ± 0.6	7.1 ± 2.7
Wax ester C_{55}	2.5 ± 0.2	–	2.2 ± 0.8
Wax ester C_{56}	18.4 ± 4.6	TR	17.6 ± 1.7
Wax ester C_{58}	7.3 ± 3.1	TR	7 ± 0.3
Total Wax esters	119.9 ± 17.4	272 ± 18.7	196.2 ± 7.9
2-Pentadecanone-6,10,14-trimethyl	5.6	234 ± 6.9	14.9 ± 0.1
Phytol	–	126.1 ± 0.7	–
Branched alcohol 1	–	183.5 ± 2.6	–
Total 'other' compounds	5.6	543.6 ± 10.2	14.9 ± 0.1

and tocopherols among other compounds. Triterpenoids were only found to be present in the leaf waxes.

Previous studies have demonstrated that sugarcane wax is predominantly composed of long-chain aldehydes (Asikin et al., 2012; Lamberton and Redcliffe, 1960; Nuissier et al., 2002; Purcell et al., 2005; Rutherford and van Staden, 1996). These results are consistent for those obtained for rind wax, however, only small quantities of long-chain fatty aldehydes were detected in the leaf wax. The main group of compounds found in the leaf wax were found to be triterpenoids (169 ± 6 mg/g of wax), followed by long-chain fatty acids and sterols. The triterpenoids identified within the leaf residues were found to be crusgallin, arundoin, cylindrin, simiarenol and friedelin.

Triterpenoids are high-value compounds, as they are known to have medicinal and pharmacological applications. It has been demonstrated that friedelin, as well as its derivatives, have a variety of properties including analgesia and anti-inflammatory abilities, anti-cancer activity, anti-bacterial activity and vascularising agent (Frame, 2003; Moiteiro et al., 2001, 2004; Nakamura et al., 1997; Pires et al., 2009; Zhang et al., 2006). Furthermore, it has the potential to be utilized in pharmaceutical products or in functionalized food for treating cardiovascular and cerebrovascular tumors and other diseases (Zhang et al., 2006).

Long-chain fatty acids were also present in considerable quantities. Saturated fatty acids having chain lengths varying from C_6 to C_{30} were detected. Odd-chain length fatty acids were detected in the extractives albeit in smaller quantities. Potential applications for saturated fatty acids are their use in soaps, detergents, polishes/cleaning products and lubricating grease (Hill, 2000). In contrast to the rind wax, the leaf wax consists of unsaturated fatty acids linolenic acid ($C_{18:3}$), linoleic acid ($C_{18:2}$) and oleic acid ($C_{18:1}$). Polyunsaturated fatty acids are known to have a hypocholesterolemic effect on serum cholesterol in humans (Ahrens Jun et al., 1957; Gill and Valivety, 1997; Horrobin and Huang, 1987; Shepherd et al., 1978). Other studies have also shown that an α-linolenic acid-rich diet has significant cardioprotective effects. Furthermore, linoleic acid, α-linolenic acid and other polyunsaturated fatty acids could be used as platform molecules to produce a variety of bio-transformation products that can be used for a variety of applications (de Lorgeril et al., 1994).

The phytosterols stigmasterol (29 ± 1.7 mg/g of wax) and β-sitosterol (39 ± 5.3 mg/g of wax) were detected in appreciable

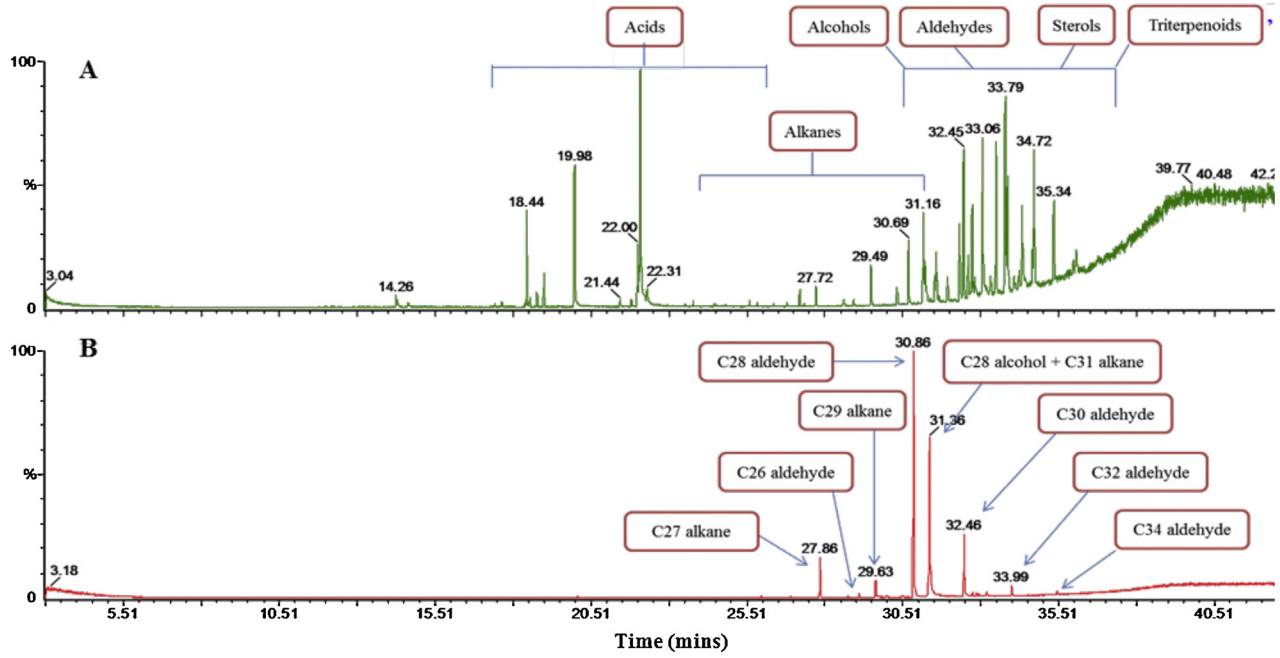

Fig. 4. Comparison of GC–MS chromatograms of unsilylated (A) Leaf wax (B) Rind wax.

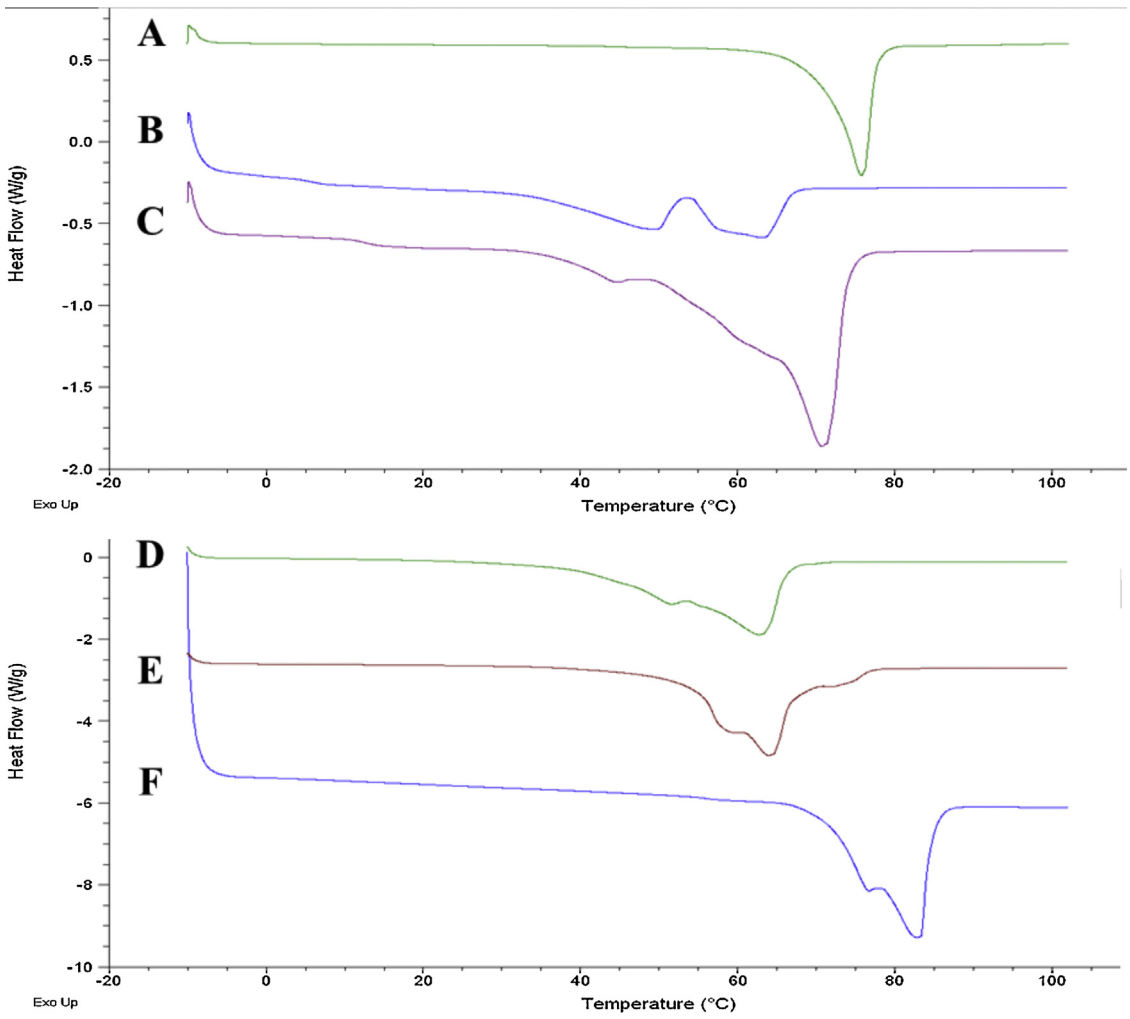

Fig.5. DSC thermograms (second heating) of (A) Sugarcane rind wax (B) Sugarcane leaf wax (C) Sugarcane bagasse wax (D) Commercial beeswax (E) Commercial candelila wax (F) Commercial carnauba wax.

quantities in the leaf wax. Phytosterols are of particular interest as they have a variety of potential high value biological and physiological applications. They are widely known to act as efficient anticancer compounds and their involvement in cholesterol metabolism and atherosclerosis has been well documented (Bradford and Awad, 2007; De Stefani et al., 2000; McCann et al., 2003; Moghadasian and Frohlich, 1999; Shimizu et al., 1991). β-sitosterol may also have a possible preventative role in patients suffering from benign prostatic hypertrophy (BPH) (Berges et al., 1995; Klippel et al., 1997).

Other compounds detected in the leaf were phytol, tocopherol (vitamin E) and phylloquinone (vitamin K_1). Phytol is of considerable interest as it is an extensively used fragrance material. It is used in a number of applications, including fine fragrances, cosmetics, toilet soap, shampoos and other toiletries, detergents and household cleaners (McGinty et al., 2010). Vitamin K_1 has interesting medicinal properties, where it has been shown to reduce postmenopausal bone loss in patients (Braam et al., 2003).

Limited work has been carried out on the full characterisation of the sugarcane leaf wax and no previous studies have been reported that extract valuable products from leaf residues using $scCO_2$. Specifically, methyl ether triterpenoid structure elucidation in sugarcane leaves has been carried out, but the wax as a whole has not been fully characterized (Bryce et al., 1967; Smith and Martin-Smith, 1978).

The exploitation of sugarcane leaf residues for wax extraction has previously not been considered. The wax from leaf residues is very different to the sugarcane wax reported from other sugarcane waste in literature, in that it has very small amounts of long-chain aldehydes and policosanols, but has appreciable amounts of triterpenoids (169 ± 6 mg/g of wax). The high amounts of triterpenoids combined with an increase in the amount of leaf residues due to green harvesting means that there is a steady source of high-value triterpenoids.

The profile of the bagasse wax had regions similar to the rind extract and other regions similar to the leaf wax. Long-chain fatty aldehydes (24.4% of total composition) and fatty alcohols (17.2% of total composition) were the dominant groups of compounds with 1-octacosanol (135 ± 1.3 mg/g of wax) and octacosanal (152 ± 1.1 mg/g of wax) predominating. However, like the leaf wax there were also appreciable quantities of phytosterols with campesterol, stigmasterol and β-sitosterol detected.

Sugarcane bagasse had the highest amount of wax esters present (37 ± 1.5 mg/g of wax). Wax esters are highly sought after for many valuable applications ranging from cosmetics to hard wax polishes, lubricants, coatings and plasticisers (Gunawan et al., 2005).

Fig. 5 compares the DSC thermograms of the sugarcane waxes with other commercially available natural waxes.

It is interesting to note that there is an appreciable difference between the DSC thermogram of the leaf wax (B), the sugarcane rind wax (A) and bagasse wax (C). Since natural waxes comprise a

complex mixture of long-chain hydrophobic compounds, it is rare to find clearly defined peaks for them (Ritter et al., 2001). This appears to be the case for the sugarcane leaf wax, which has a broad DSC trace with two endothermic minima centered at around 48 °C and 63 °C, respectively, as a result of the large variation in the family of compounds constituting the wax. The second DSC transition is similar to that found in the commercial beeswax (63 °C) and candelila wax (64 °C).

In contrast, the sugarcane rind has a well-defined narrow peak, with a minimum at 76 °C. This is potentially attributed to the high abundance of specific types of compounds (≈83% of the rind wax is composed of long-chain alcohols and long-chain aldehydes) which would therefore give a sharper more clearly-defined thermal transition when compared to the leaf wax. The sugarcane rind wax has the highest melting profile as a result of the large abundance of long-chain fatty aldehydes present. Furthermore, the DSC trace shows that there is only one transition when compared to the other types of sugarcane wax as well as the commercial waxes (the other waxes all have minor transitions as a result of broader range of compounds present). The bagasse wax has an endothermic minimum at 71 °C.

The difference in wax composition, together with the variation in the DSC traces, suggests that waxes obtained from various parts of the plant could be utilized in different applications. The sugarcane leaves have a broader range of compounds and have a high abundance of triterpenoids suggesting that these could be utilized in nutraceutical or pharmacological applications. The DSC profile of the rind wax exhibits a slightly lower melting compared to that of the carnauba wax (83 °C) and could be used in similar applications, such as automobile and instrument polishes. Furthermore, the high abundance of policosanols in the rind and bagasse wax would make them an ideal source for the production of cholesterol-reducing nutraceuticals.

It is important to assess the supercritical extraction of sugarcane waxes by comparison with conventional extraction solvents. Conventional extraction techniques, such as soxhlet extraction, are still considered to be a benchmark to compare other extraction techniques (Azmir et al., 2013). Hexane, which is the most commonly used extraction solvent, is petroleum-based, a hazardous air pollutant (as listed by the US EPA in the Clean Air Act 1990) and a neurotoxin having severe adverse effects on the nervous system (Deswarte et al., 2006).

Soxhlet extractions were carried out on the leaf and rind biomass using hexane and the results were directly compared to those obtained for the supercritical extractions.

It was found that the same types of compounds were extracted for both types of extraction techniques, however there was a variation in the distribution and quantities of compounds extracted. Fig. 6 shows that for most family of compounds, $scCO_2$ extracts larger quantities of molecules than hexane. This phenomenon could be due to the entrainer effect of solute molecules in the supercritical fluid phase, whereby solute molecules act as co-solvents enhancing the solubility of other less soluble compounds (Dobbs et al., 1987). Therefore, as compounds are incorporated into the supercritical phase, the solvation properties of $scCO_2$ change significantly resulting in an enhanced extraction of compounds (Dobbs et al., 1987).

There was a significant variation in the quantities of fatty acids extracted when using $scCO_2$ and hexane. Significantly larger quantities of these molecules were extracted with $scCO_2$ (1515 ± 16.1 μg/g of dry plant) when compared to hexane (868.7 ± 53.5 μg/g of dry plant). One of the reasons for this is attributed to the much higher concentrations of unsaturated fatty acids (linolenic acid, linoleic acid and oleic acid) present in the $scCO_2$ wax extract (421.2 ± 4.5 μg/g of dry plant compared to 99.3 ± 21.9 μg/g of dry plant for the hexane extract).

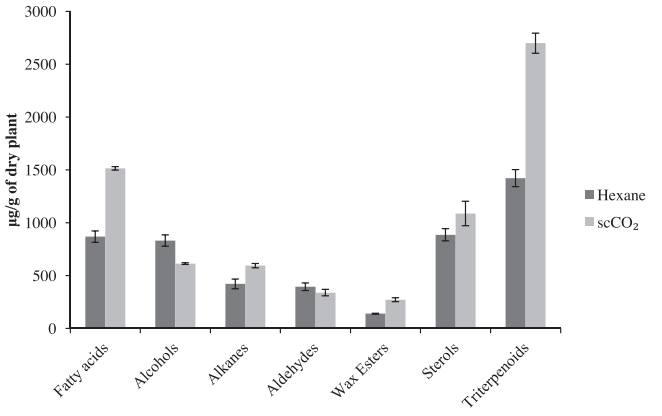

Fig. 6. Composition of waxes extracted from leaf residues using hexane and $scCO_2$ in μg/g of dry plant.

Similar results to this study were obtained by Bernardo-Gil et al. when extracting unsaturated fatty acids from hazelnut oil, where a higher concentration of unsaturated fatty acids was obtained when using $scCO_2$ when compared to hexane (Bernardo-Gil et al., 2002).

In contrast to the fatty acid concentration, the total *n*-policosanol content was smaller in the $scCO_2$ extracts when compared to hexane extracts with total concentrations of 613.7 ± 8 μg/g of dry plant and 831.9 ± 59.3 μg/g of dry plant respectively. Higher quantities of 1-hexacosanol were found in the supercritical extracts. Similar results were obtained for the long-chain fatty aldehydes, with hexane exhibiting greater extraction yields.

Larger quantities of *n*-alkanes were present in the $scCO_2$ extracts (594.3 ± 20.7 μg/g of dry plant) compared to the hexane extracts (421.8 ± 46.2 μg/g of dry plant). Extraction of wax esters was greater with $scCO_2$ extraction (272 ± 18.7 μg/g of dry plant) than hexane extraction (140 ± 4 μg/g of dry plant).

The total phytosterol concentration was the higher in the $scCO_2$ wax. Studies have shown that higher concentrations of phytosterol can be extracted with $scCO_2$ than with soxhlet extractions using DCM or recirculated hexane extraction (Castola et al., 2005; Wang et al., 2007). The triterpenoid profile of the $scCO_2$ extract was found to be drastically different to that of the hexane soxhlet. Significantly larger amounts of triterpenoids were extracted when using $scCO_2$ than hexane, with total concentrations of 2698.8 ± 95.3 μg/g of dry plant in the $scCO_2$ extract and 1422.2 ± 80.5 μg/g of dry plant in the hexane extract.

Similar results were obtained from the rind wax, as shown in Fig. 7, with larger amounts of *n*-policosanols and long chain fatty

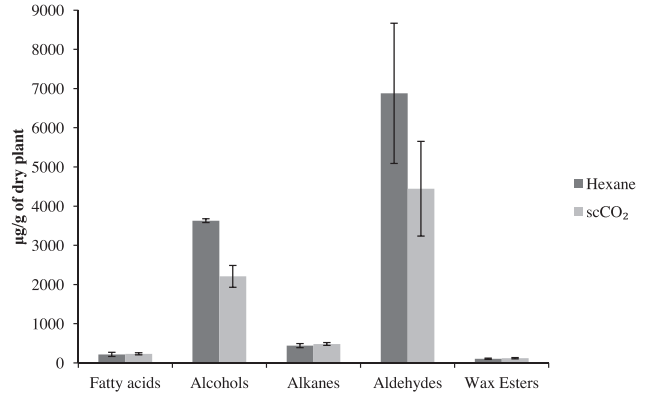

Fig. 7. Composition of waxes extracted from sugarcane rind using hexane and $scCO_2$ in μg/g of dry plant.

aldehydes in the hexane extracts, while slightly higher concentrations of long-chain fatty acids, *n*-alkanes, and wax esters in the $scCO_2$ extracts.

$ScCO_2$ extraction has a number of advantages over conventional soxhlet extraction. A large number of these compounds have nutraceutical and medicinal properties and are often incorporated in number of food and drink products. CO_2 has the added benefit of potentially being a food-grade solvent. As stated previously, hexane is not only petroleum-based, but is also a hazardous air pollutant (as listed by the US EPA in the Clean Air Act, 1990) and a neurotoxin (DeSimone, 2002; Schaumburg and Spencer, 1976).

Furthermore, the supercritical extraction of the waxes from the sugarcane waste can be utilized as a pre-treatment step within a biorefinery. Sugarcane bagasse and rind are already utilized as a large scale fermentation feedstock for ethanol production. Supercritical extraction of these waxes has no detrimental effects on the biomass and studies have shown that the $scCO_2$ extraction has a positive effect on the downstream processing of biomass, enhancing saccharification of lignocellulose to sugars for the production of surfactants and biofuels (Attard et al., 2015). Therefore, supercritical extraction further generates added-value to this agricultural waste.

4. Conclusions

The supercritical extraction of waxes from different types of sugarcane waste (rind, leaves and bagasse) has been successfully carried out in order to obtain high-quality compounds which would potentially add value to this waste biomass. This work demonstrates that different botanical components of sugarcane waste give rise to waxes with substantially different compositions. The rind wax provides substantially large quantities of *n*-policosanols and long-chain fatty aldehydes (83% of the total wax composition) while the leaf residues wax can be an excellent source of triterpenoids (169 mg/g of wax) along with unsaturated and saturated fatty acids and phytosterols. The exploitation of leaf residues as a potential source of wax has not been previously considered. Bagasse wax provides the highest amount of wax esters. These results, along with the DSC traces, have shown that these different waxes can therefore be used in different applications. Furthermore, the use of $scCO_2$ could allow the direct use of molecules within food or drink applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2015.05.077

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