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Supercritical CO_2 Extraction of Lipophilic Molecules from Sugarcane Straw

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The extraction of lipophilic molecules from sugarcane straw is an essential component as a part of a holistic biorefinery. Bearing this in mind, this work focuses on supercritical CO_2 ($ScCO_2$) extraction assisted by ultrasound as a clean efficient process. The effects of operating parameters temperature (40 - 60 °C) and ultrasound power (400 W – 800 W) on the $ScCO_2$ global yield and Field emission scanning electron microscopy (FESEM) on solid residue remaining were studied. The maximum global yield of the extracts (0.90 ± 0.10 g extract/ g straw) was obtained at 60 ºC, and 800 W. FESEM was used to analyze the solid residue remaining with and without ultrasound and showed that ultrasound disturbs the cell walls, enhancing the release of the extractable compounds. Statistical analysis showed that the temperature was the parameter that influenced the global yield of the extracts. This technology crucially improves the downstream conversion since $ScCO_2$ not only can be used as an extraction solvent, but also for the pre-treatment. Sugarcane straw lipophilic molecules can be useful in a host of applications, ranging from cosmetics to hard wax polishes, lubricants, coatings, and plasticizers.

1. Introduction

Sugarcane is a significant agriculture activity in the world, especially in Brazil, where the production of sugarcane in Brazil reached 620 Mt in the 2018/2019 harvest. According to UNICA (Sugarcane Industry Union), the harvesting sugarcane produces considerable quantities of straw, for each ton of sugarcane processed, an estimated 140 kg of straw are generated (Lachos-Perez et al., 2017). Sugarcane straw is basically composed of cellulose, hemicellulose, and lignin, but also contains low content of lipophilic molecules (ca. 1–2% by weight).

The composition of lipids in plants such as sugarcane has been the target of several works, including triterpenoids and steroids (Feng et al., 2014a; Feng et al., 2014b) or aliphatic components such as long-chain alcohols (policosanol) and aldehydes (Asikin et al., 2012; Purcell et al., 2005) have already been identified. The extraction of lipids from biomass is usually performed using conventional organic solvents such as dichloromethane, chloroform, hexane, acetone, and toluene, having as bias the generation of toxic waste, which is related to environmental and/or toxicological problems (Deswarte et al., 2006). In this context, a suitable alternative solvent for the extraction of natural products is $scCO_2$, since CO_2 is non-flammable, non-toxic and widely available. In addition, CO_2 is a recyclable and unregulated solvent (Subramaniam et al., 1997). Additionally, it is already available in the ethanol mill from sugar cane as a by-product from fermentation.

$ScCO_2$ assisted by ultrasound not only is used in order to improve the extraction efficiency but also for the pre-treatment of lignocellulosic material (Barrales et al., 2015; Pasquel Reátegui et al., 2014). Ultrasonic waves produce compression and decompression cycles. Such cycles may lead to the rupture of cell walls of a vegetal substrate, promoting the penetration of the solvent and mass transfer. On the other hand $scCO_2$ remove the lipid layer that covers the surface of the vegetable matrix, as well as small recalcitrant biomass

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pores that are easily penetrated by CO_2 at high pressures or that cause structural change and facilitate the downstream process of biomass conversion.
The objective of this work was to investigate the effects of temperature and ultrasonic waves on the $scCO_2$ global yield of sugarcane straw as well as to evaluate the effect of the ultrasonic waves on the material *in nature* and sugarcane straw residue after the extraction by FESEM.

2. Material and methods

2.1 Raw material

Sugarcane straw (Saccharum officinarum Linnaeus) was supplied by the sugar and ethanol plant Ester located in Cosmópolis, São Paulo, Brazil. Sugarcane straw was ground (batches of 10 g in 20 s) in a knife mill (Marconi, model MA 340, Brazil) coupled to an induction motor of 3800 rpm, and separated by sieving using sieves with meshes of 16, 24, 32, 48, 80 and 100 on the Tyler series and then obtained the mean diameter between sieves of 303 µm.

2.2 Global yield

For each extraction experiment (Soxhlet, $scCO_2$ with and without ultrasound), the extraction global yield (X_0) was calculated according to Eq(1), which relates the total extract mass ($m_{extract}$) and the sample mass (F) in dry basis.

$$X_0\left(\%\right)=\frac{m_{ext}}{F}\cdot 100 \tag{1}$$

2.3 Soxhlet extraction

The Soxhlet method was selected as a conventional extraction technique, using hexane as solvent, due to its non-polar affinity, which is suitable for lipid extraction. For each extraction 5 g of dried sample were packed in filter paper and inserted in the Soxhlet extractor. Hexane (0.15 L) was added, and the system was heated until boiling. Reflux was kept for 4 h, then the solvent was evaporated under vacuum (at 35 °C), and the recovered extract was weighed and stored under freezing (−18 °C) until further analyses. The Soxhlet extractions were performed in triplicates.

2.4 Supercritical extraction assisted by ultrasound

The $scCO_2$ experiments were performed in an ultrasound-assisted supercritical fluid extraction ($scCO_2$ + US) unit, which consists of a 0.3 L extraction column; a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany); two thermostatic baths (model MA184, Marconi, Campinas, Brazil) to control the temperature of CO_2 at the pump inlet and $scCO_2$ temperature; a flow totalizer and manometers. An ultrasound probe with a 13 mm titanium end, coupled to a transducer (Unique Group, model DES500, Campinas, Brazil) is installed on the upper end of the extraction cell and is operated from an ultrasound generator that can operate from 10 to 99% of its total power (800 W). Figure 1 illustrates the $scCO_2$ + US unit, with special focus on the SFE + US bed.
The mass of sugarcane straw used in each extraction was 10 g. The $scCO_2$ procedure was composed of an initial static extraction time of 20 min for all of the experiments, and after that, a dynamic extraction time of 120 min for the global yield experiments. The mass ratio between solvent and sugarcane straw (S/F) was kept constant at 126 ± 2kg CO_2/kg straw in the global yield experiments. This ratio was assured by keeping constant the solvent flow rate (1.75 × 10−4 kg/s) and extraction time (120 min without including the static time). The application of ultrasound was implemented only during the static time of 20 min.
The experimental conditions are expressed in Table 1, and they were performed in duplicates to ensure reproducibility. Pressure has been selected based on the work of Attard (Attard et al., 2018). Temperature range has been selected by previous assays with the same raw matter, considering as a working range temperature from 40 to 60 °C. Regarding the ultrasound power, the maximum reachable range of values with the equipment was used (from 0 to 800 W).

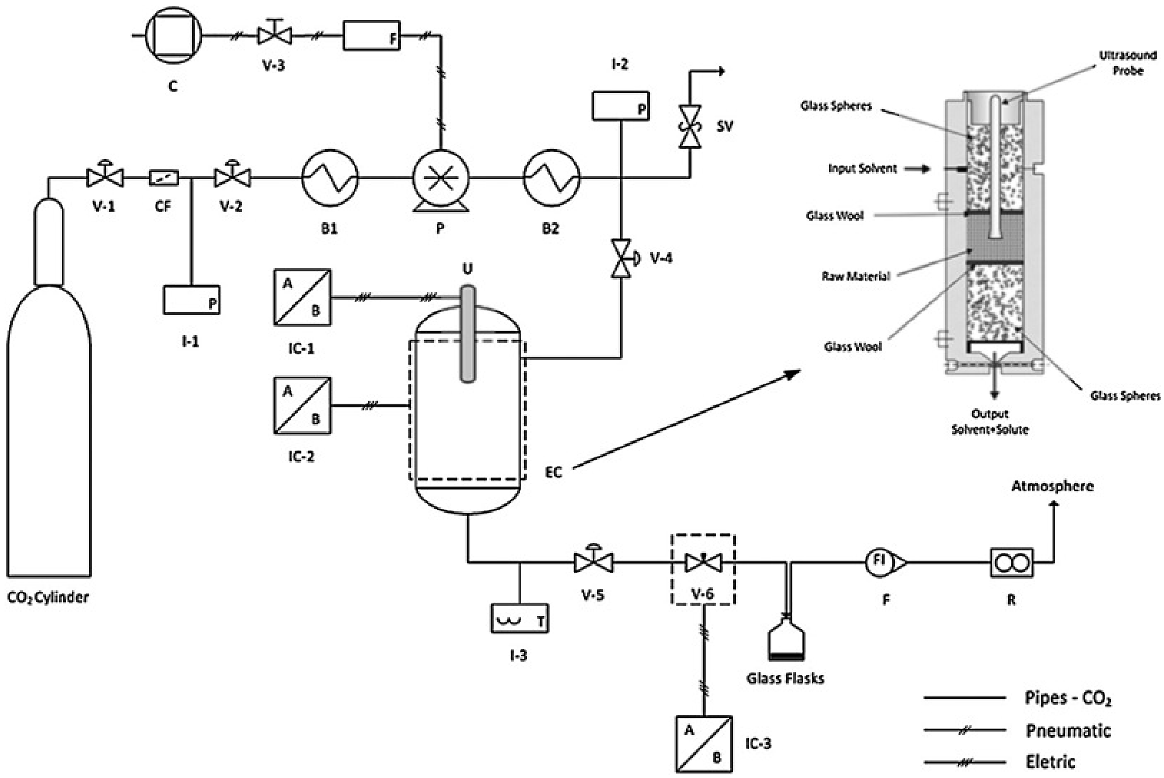

Figure 1. Diagram of the SFE + US unit: V-1–V-5–control valves; V-6–micrometer valve; SV – safety valve; C – compressor; F – compressed air filter; CF – CO2 filter; B1–cooling bath; P – pump; B2–heating bath; I-1 and I-2–pressure indicators; I-3–temperature indicator; IC-1, IC-2 and IC-3–indicators and controllers of ultrasound power, temperature of extraction column and temperature of micrometer valve, respectively; U – ultrasound probe; R – flow totalizer; F – flow meter; EC –extraction column and internal configuration of the extraction bed of 300 mL for SFE + US used in the experiments.

2.5 Statistical analysis

To prove the model significance with a confidence level of 95%, analysis of variance (ANOVA) has been performed using the software Statistica 7. All the experiments described in this section were analyzed through the Tukey's test, with a significance of 5%, to determine if there were significant differences between the different treatments.

2.6 Field emission scanning electron microscopy (FESEM)

The microstructure of the surface of sugarcane straw residue after $scCO_2$ + US and in nature were analyzed. The FESEM equipment was a Sputter Coater EMITECH, Model: K450 (Kent, Reino Unido). Prior to analysis, the samples were coated with gold in a SCD 050 splutter coater (Oerlikon-Balzers, Balzers, Liechtenstein). Analyses of the sample surfaces were performed under vacuum, using a 15 kV acceleration voltage.

3. Results

3.1 Global yield

Preliminary $scCO_2$ tests were performed on the dried and milled raw material in order to fix the extraction time. Next, the extraction bed was prepared, as described in Section 2.4. Table 1 shows the extract yields (X_0) obtained in the $scCO_2$ + US. A moderate increase in X_0 is observed when ultrasound was applied. The application of ultrasound can produce a cavitation effect near the cell walls (Barrales et al., 2015; Pasquel Reátegui et al., 2014), and also the release of soluble material onto the sample surface, leading to higher yields. Therefore, the extract yields achieved by $scCO_2$ + US are quite higher than those of $scCO_2$ without ultrasound at the same temperatures.

Table 1: Global yield of $scCO_2$of sugarcane straw oil without and with ultrasound at 25 MPa.

Codified variables		Real variable		X_0(%)	CO_2 density (kg/m^3)
X_1	X_2	T (ºC)	U (W)		
		40	0	0.68± 0.06	879.49
		40	400	0.62 ± 0.01	879.49
		40	800	0.72 ± 0.07	879.49
		50	0	0.79± 0.03	834.19
		50	400	0.54± 0.11	834.19
		50	800	0,79± 0.03	834.19
		60	0	0.75 ± 0.01	786.55
		60	400	0.81 ± 0.16	786.55
		60	800	0.90 ± 0.10	786.55
Soxhlet Hexane				1.24 ± 0.10	-

The highest values of CO_2 density achieved the highest yield, which may explain the increase of its solvation power. The highest $scCO_2$ global yield (0.9 ± 0.1%) was achieved at 60 °C, and ultrasound power of 800 W. According to Balachandran et al. (2006), the increase of global yield can be attributed to the high extraction rates achieved in the process assisted by ultrasound. The application of ultrasound causes ruptures in the vegetal structure, promoting the release of compounds that were not formerly available, so the yield can be increased. Another possible effect of ultrasound in $scCO_2$ is the release of extractable material onto the sample's surface (Balachandran et al., 2006). In this case, the desorption of solute from the substrate to the solvent is enhanced, increasing mass transfer and yield.

Comparing $scCO_2$ + US with Soxhlet extraction, it can be noted in Table 1 that Soxhlet with hexane achieved higher yields, which can be attributed by the solvent recycle and solvent/solute interactions of Soxhlet, which contribute to enhancing the solubility of most compounds of the sample. Moreover, the longer extraction times and solvent to solid proportion also help to increasethe global yield. Soxhlet extraction can be useful, but present disadvantages such as high required volume of solvent, long process times, and contamination of the extract with residual solvent (Lachos-Perez et al., 2018; Pasquel Reátegui et al., 2014).

Table 2. Table SI – 1. Analysis of variance for the influence of parameters (Temperature and Ultrasound) on the global yield (X_0)

	Sum of Squares	df	Mean square	F-value	p-Value Prob>F
Source					
Temperature (linear)	0.065520	1	0.065520	5.440660	0.044563
Temperature (quadratic)	0.015654	1	0.015654	1.299899	0.283664
Ultrasound (linear)	0.003595	1	0.003595	0.298518	0.598094
Ultrasound (quadratic)	0.042361	1	0.042361	3.517552	0.093473
Pure error	0.108383	9	0.012043		
Corrected total	0.287414	13			

Experimental results of the analysis of variance (ANOVA) were fitted by mean square method, and the response model coefficients analyzed using F-test are presented in Table 2. The only factor with a significant effect was temperature, showing a linear effect over the global yield. As temperature determines the $SCCO_2$ density, it was expected to have a significant effect. On the other side, ultrasound power seems to have no significant effect on the global yield. However, this result might be mascaraed by the significant effect of temperature over the process.

3.2 FESEM

Figure 2 shows FESEM images of the sugarcane straw before extraction – in nature (a), and after Soxhlet (b) and $scCO_2$ extraction without (c) and with ultrasound (d). It can be observed that samples treated only with Soxhlet extraction present not quite difference and a much more homogeneous and cleaner surface when compared with material in nature (Figure. 2 a-b), indicating that low-pressure extraction did not cause important morphological changes.

In contrast, samples exposed to ultrasound exhibit an irregular surface, covered by particle fragments; ultrasonic vibration effectively changes the surface of the sugarcane straw remaining residue particles, and the surfaces of the particles are generally smooth, without striations or pores, both before and after the extracting processes.
It is essential to highlight that the observed effect of the extraction process is limited to the removal of the superficial tissue that covers the vegetable matrix and of the broken material that is deposed on the surface. When higher magnification images are taken (not shown in this work), it is noted that the morphology of the sugarcane straw residue was clearly influenced by $scCO_2$ + US process. The relatively flat surface observed in the untreated sample appears to become more degraded and covered by particulate material.

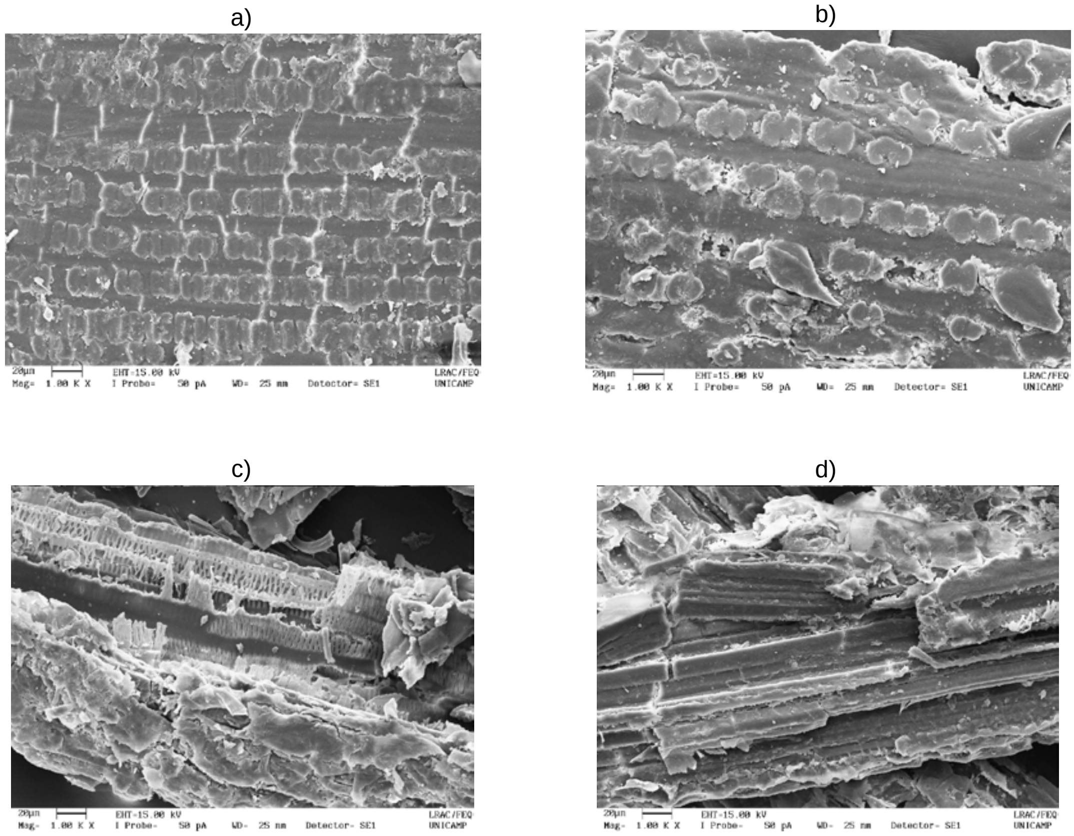

Figure 2. FESEM images of sugarcane straw particles: (a) unextracted; (b) after Soxhlet extraction with hexane; (c) after $scCO_2$ extraction without ultrasound and (d) after $scCO_2$ extraction without assisted by ultrasound.

4. Conclusions

Lipophilic molecules from sugarcane straw were extracted using $scCO_2$ assisted by ultrasound. The application of ultrasound helped to increase the global yield when compared to $scCO_2$ without ultrasound.The enhancement of mass transfer with ultrasound is caused by changes in the structure of the substrate, which were observed by the FESEM image analyses. Specifically, the increase of particles stuck onto the surface of sugarcane straw may have contributed to reducing the barriers to both solvent and extractable compounds. FESEM analysis from remaining residual showed that $scCO_2$+US remove the lipid layer of the vegetable matrix, as well as small recalcitrant biomass pores that are easily penetrated by CO_2 at high pressures, or that cause structural change and facilitate the downstream process of biomass conversion. The results are promising results, although analysis of the lipophilic profile of the extract by chromatography should be done to find future applications in the extracts. Further, a technical and economic evaluation of the process and scale-up should be studied.

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