Understanding the extraction capabilities of the Helix Supercritical Fluid System

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Abstract

Supercritical fluids extraction is a separation technique, which has higher yields and lower extraction times than conventional extraction techniques. Moreover, due to the general use of carbon dioxide a supercritical medium there is also a reduction in the use of toxic organic solvents.

Although there are commercial supercritical fluid extraction systems, these systems are not standardized, and each different system has its own particularities. At Universidad EAFIT there is a new supercritical fluid equipment, a Helix model of Applied Separations, that has not been thoroughly tested for its operation.

The extraction of glycosides from stevia was used as a model for understanding the effect of different parameters, such as pressure, temperature, CO_2 flow, matrix load and extraction time, on the operation the Helix system at EAFIT University. The effect of the changes in the variables was evaluated by calculating extraction yield and by characterization of the extract. The obtained extract was characterized by IR and HPLC to verify the extraction of glycosides.

As a result of this project an operation manual for the equipment was written.

Pressure seems to be the more influential operating parameter, both for the matrix extraction and equipment performance. Pressure and temperature have repercussions on other process variables as preparing, extraction and cleaning time or CO_2 consumption. However, all the evaluated operating parameters represent a contribution for the equipment performance and their understanding is essential for improved capability of Helix system.

A maximum yield of 1.4% was obtained. Pressure was the most important variable on the glycosides extraction yield, while the temperature did not have a noticeable effect on the yield. Similarly, the use of cosolvent remarkably improved the performance at same conditions of pressure and temperature. An approximate extraction time of 120 minutes was required.

1. Introduction

Supercritical fluids are widely used as solvents because of their good mass transfer characteristics and solvent strength. Their application areas include impregnation and cleaning, multistage counter-current separation, particle formation, coating, and reactive systems such as hydrogenation, biomass gasification, and supercritical water oxidation [1].

Carbon dioxide is the main supercritical fluid because its low cost, environmentally friendly (innocuous to human health and to the environment) and it is generally recognized as safe [2]. In addition, CO_2 has moderate critical conditions compared to other substances, and is gaseous at room temperature and pressure, which makes extract recovery a simple process.

Supercritical fluids extraction (SFE) is applied to obtain extracts enriched with compounds of interest, typically used in food, cosmetics and pharmaceuticals, because it can reach high selectivity and prevent traces of the solvent in the final extract [3], which are important characteristics for these highly regulated products.

Also, SFE compared to conventional solvent extraction presents higher yields and lower extraction times than conventional extraction techniques, besides not requiring the use of toxic organic solvents. [4][2].

There are a large number of reviews about SFE fundamentals, experimental design and specific applications on extraction of metals as complexes [5], vegetable matrices [6], functional ingredients from natural sources [7], decontamination of hazardous substances [8]. Supercritical CO_2 can be specially applied in food processing [9], with relevance in the fractionation, extraction, microencapsulation, pasteurization, sterilization, and chromatograph techniques, among others [10–13]. Moreover, several technological advancements have been performed in combination with SFE methodology to improve extraction yield and selectivity of bioactive compounds [14].

Modifiers have been applied to enhance the extraction of the target compound. Among the most commonly used modifiers are: ethanol [15–17], propane [18] and water. Ethanol is usually used to aid in the extraction of polar compounds with a proportion that could vary between 5% and 30% of the inlet CO_2.

Extraction conditions like temperature, pressure and time have also been studied. For example, bioactive flavonoid compounds were obtained from spearmint (*Mentha spicata L.*) leaves by Bimakr et al. [19]. They evaluated the effect of temperature, pressure and dynamic extraction time on the CO_2 SFE. Optimal conditions were 60°C, 200 bars and 60 min (in a 500 ml extraction vessel and a $CO_2\ flow\ of\ 5 g/$min), concluding that temperature increases solute solubility, and pressure increase fluid density, altering solute solubility. Gomes et al. [20] produced rose geranium oil by SFE evaluating different extraction conditions, and found that time does not affect significantly extraction yield and composition after 5 minutes (in a 1L extractor at 1.6 kg CO_2/h) and that a higher recovery of volatile fractions and a lower recovery of non-volatile fractions are obtained at high pressures. Another example, is the extraction of high activity antioxidant compounds from peach palm pulp (*Bactris gaspae*s) carried out by Espinosa-Pardo et al. [21], who studied pressure and temperature, obtaining optimal conditions of 40°C and 300 bar. The optimal extraction conditions should be studied for different extracts due to biomass origin, morphological characteristics as well as different target compounds.

Hinojosa et al. [22] evaluated supercritical CO_2 extraction to obtain the glycosides from leaves of Stevia Rebaudiana Bertoni, studying temperature, pressure, extraction time and the presence or absence of the co-solvent incorporated in different proportions to determine the effect on yield. The pressure was the factor that favored the extraction. They concluded that supercritical CO2 was not efficient to extract Stevia sweeteners.

SFE studies in Colombia [23–30], have been aimed to more efficient use of natural resources, being representative those relates to extraction of volatile extracts (aromas), from fruit products and aromatic plants, or the use of agroindustry residues.

Although they are widely used SFE equipment are not standardized. There are a lot of different systems and each one has its own particularities. SFE systems in Brazil and Latin America have small size compared to industrials units used in Asia, North America and Europe [31]. These small semi-industrial units should be used to scale up the process, by means of giving important operation data in order to select process conditions.

Cezar et al. [31] described the setup of a laboratory unit for supercritical fluid extraction. They validated the plant with commercial units using extraction of annatto seeds and made a comparison between the costs of acquisition and setup of the laboratory equipment for extraction with supercritical fluid, demonstrating that the design of the SFE unit resulted in lower costs than the commercial unit. The reported construction of this SFE equipment can be used for construction of pilot-scale applications.

Despite most SFE applications are related to the combination of different technologies to achieve higher extraction efficiency there still is margin for further improvements, and additional combinations with all the enhancements should be considered [14]. The interest in SFE is not only as an analytical tool but also for process development, to lead to higher extraction yields and greater selectivity of compounds with significant interest to the industry. Therefore, it is important to improve equipment capabilities by means understanding the process and having good operating practices.

For the aforementioned applications to succeed and the university implement sustainable uses of Colombian biodiversity for the natural products and food industries, the properties of supercritical fluids in combination with the materials processed must be clearly determined. The phase equilibrium at supercritical conditions is highly sensitive to modifications in temperature and pressure [2], therefore it is important to properly understand the contribution of all variables for the equipment performance to improved capability of system and take advantage of EAFIT's Helix Supercritical Fluids System.

The aim of this work is to understand the performance of a supercritical fluid extraction equipment, by means different experiments than will allow to understand the effect of equipment operation parameters and develop

criteria to validate the operation of the system. This study is required in order to understand the performance of a SFE equipment. Although the extraction of glycosides from stevia is selected for performing this study, the main objective of this work is to validate the equipment operation and not optimizing the yield or quality of the extract.

2. Methodology:

Organic matrix selection and pre-treatment

Stevia rebaudiana was selected for experiments according to availability. Stevia was cultivated at 1300 MASL in Barbosa, Antioquia. Dry Stevia leaves were subject to size reduction process to increase the transfer of matter [32], using a home mincer and reaching a mean size of 0.8 cm.

Gliricidia Sepium obtained from Lagos del Dulcino, Santa Marta – Magdalena, at o MASL, was also tested in experiments. Gliricidia was also subject to a size reduction process, but in this case using a grinding machine to reach a size of less than 600 microns.

Supercritical fluid extraction

The extraction by supercritical carbon dioxide was performed using a Helix Supercritical Fluid System (Applied Separations, USA). The dry matrix was weighed and loaded into a 1000 ml extraction vessel, placing glass wool (2g approx..) at both the ends to prevent leakage from vessel. Compressed CO_2 was added from the bottom of the extraction vessel and heated. Total extraction time was varied among experiments. For experiment 5 (**E5**), a 300 ml separator vessel was also connected (at 30 bars and 35°C) in search of separating different compounds (fractionation). The outlet valve temperature was set at a high temperature to prevent plugging of valve because of cooled extracts due to expansion of compressed CO_2. The flow rate varied among experiments, but it was constant during each experiment.

The initial extraction conditions for Stevia were taken from a previous study [22] and from preliminary extractions. Experiments were performed at conditions showed in **Table 1**.

For the co-solvent a Series 1500 Digital HPLC pump was utilized to add ethanol at a desired system percentage. The filing time for the percentage desired (X) was calculated first determining the total amount of CO_2 inside the 1000 ml extraction vessel (m_{CO_2})

$$m_{CO_2} = \frac{44.01 * P_{Ext\ vessel} * 1}{T_{Ext\ vessel} * 0.08314 * Z} \qquad (1)$$

Where, 44.01 is carbon dioxide molecular weight; $P_{Ext\ vessel}$, extraction pressure; $T_{Ext\ vessel}$ extraction temperature; 1, extraction vessel volume (L); 0.08314, universal gas constant ($bar * L/mol * K$); and Z, the compressibility factor, calculated by Peng Robinson EOS. The mass of ethanol (m_{Etanol}) required was found from the percentage and the mass of CO2, $m_{Ethanol} = X * m_{CO_2}$. The filling time ($t_{filling}$) required for reaching the desired ethanol percentage inside the vessel was calculated as:

$$t_{filling} = \frac{m_{Ethanol}}{\rho_{Ethanol@T,P} \cdot F_{pump}} \qquad (2)$$

$\rho_{Ethanol@VT,P}$, ethanol density at the vessel conditions and F_{pump}, the maximum flow of the cosolvent pump ($12\ mL/min$). The flow of ethanol ($F_{Ethanol}$) for continuous operation can be calculated from the flow of carbon dioxide in the process (F_{CO_2}).

$$F_{Ethanol} = \frac{F_{CO_2} * \rho_{CO_2@25°C} * X}{\rho_{Ethanol@25°C}} \qquad (3)$$

For a **2%** of ethanol in the extraction process on a system at 320bar, 90°C and a CO_2 flow of 1.2 L/min, a filling time of 2.07 **minute**s and an ethanol flow rate of $0.0436\ mL/min$ were obtained.

One of the most important parameters for evaluating the performance of the system is the total amount of CO_2 consumed during extraction ($m_{CO_2,consumed}$), which can be calculated as the sum of the mass of CO_2 in the extraction vessel and the mass of CO_2 in the output flow as follows:

$$m_{CO_2,consumed} = m_{CO_2} + \rho_{CO_2@out\ cond.} * F_{CO_2} * t_{ext} \qquad \textbf{(4)}$$

Where $\boldsymbol{\rho_{CO_2@out\ cond}}$ corresponds to CO_2 density at output conditions and $\boldsymbol{t_{ext}}$, to extraction time.

To simplify this calculation an excel file was developed (**See Table 4 for a link to the file**)

The weight of the extract was recorded at different time intervals during the extraction process in order to construct an extraction curve, which plots the ratio of obtained extract mass to feed mass (yield)as a function of the volume of solvent used.

To isolate the effects of the operating conditions each experiment was run using a new load of the selected matrix.

An operation manual for the equipment, that includes operating mode and security notes, was written as a result of the experiments described in this work (**See Table 4 for a link to the manual**).

Characterization of SFE extracts

The extracts obtained from supercritical CO_2 and a Soxhlet extraction with ethanol were analyzed using IR and HPLC at EAFIT's Instrumental Analysis Laboratory.

Infrared analysis was performed on a PerkinElmer FT-IR Spectrometer Spectrum Two, and results were contrasted with theorical available standards of estevioside and rebaudioside A.

For HPLC (Agilent Technologies 1200 Series), the obtained extracts were diluted on methanol. The procedure was performed on a CN column (ZORBAX Eclipse XDB-CN, 4.6x150mm, 5 micron), at 35°C and 1 mL/min flow rate, with UV detector at 210 nm. The isocratic mobile phase was Acetonitrile:Water (5:95) [33]. All samples were filtered through a 0.45 µm filter prior to use in HPLC analyses. Identification of compounds in samples was done by means of retention time and available theorical standards.

3. Results and analysis

Table 1 shows extraction yield obtained for each experiment. The maximum yield reached by CO_2 SFE was 1.4%, a low value considering that Stevia leaves accumulate around 3.8% rebaudioside A and 9.1% stevioside.

Hinojosa et al. [22] report a value of 2.89% for a 45 minutes extraction at 400 bars, 75°C and 20% co-solvent.

Maximum yield was reached at the upper values of the operating pressure and extraction time (640 bar and 363 min.) and, on the other hand, in ethanol Soxhlet extraction 2.6 times the maximum yield by SFE was obtained. This suggests, that CO_2 supercritical fluid extraction is not selective for extracting Stevia glycosides as observed by Hinojosa et al. [22].

Table 1. Conditions and results of the experiments

	E1	E2	E3	E4	E5 Sep	E6	E7	ES
Matrix	Stevia Rebaudiana	Stevia Rebaudiana	Stevia Rebaudiana	Stevia Rebaudiana	Stevia Rebaudiana	Stevia Rebaudiana	Gliricidia Sepium	Stevia Rebaudiana
Pressure (bar)	320	640	640	320	200	200	200	-
Temperature (°C)	90	90	40	90	40	40	40	-
Average flow (LPM)	2.78	1.16	1.71	1.22	2.45	2.88	2.42	-
Load (g)	88.51	56.5	53.96	52.5	56.8	56	25	38.15
Co-solvent proportion	-	-	-	2%	-	-	-	-
Extraction time (min)	87	372	363	210	120	132	127	120
Yield (%)	**0.40**	**1.33**	**1.40**	**0.57**	**-**	**0.40**	**0.48**	**3.67**

According with **Figure 1**, that shows extraction yield as a function of CO_2 consumption, higher pressure favors extraction. Increasing pressure results in an increase in fluid density which can reached a more efficient solvent impregnation into the matrix. This result coincides with Hinojosa et al. [26] and Zarena et al. [40].

Temperature did not have an important effect on the yield. At the high pressure (640 bar), changes in temperature did not improve extraction yield (see **Figure 1**). Furthermore, low temperatures are desired for conservation of the extract and the matrix. Nevertheless, the extraction pressure and temperature and their effect on the extraction depend largely on the target compounds.

Co-solvent effect can be appreciated on **Figure 1**, at same pressure and temperature the extraction yield increased from 0.4% to 0.57%. Moreover, the kinetics of the process were largely increased, and the use of co-solvent yielded similar results to the use of higher pressure. This result can be explained because the co-solvent increases the polarity and density of the solvent, which allows more interactions with the solutes. Moreover, co-solvent induce changes in the structure of matrix by swelling it and breaking analyte-matrix bindings [34].

Although CO2 is an excellent solvent for non-polar analytes, its most frequent limitation as an analytical extraction solvent is that its polarity is often too low to obtain efficient extraction [35]. Polarity of target compounds can explain that ethanol Soxhlet extraction shows better yields than SFE, and by the same way, this can be appreciated on increasing effect of co-solvent in yield.
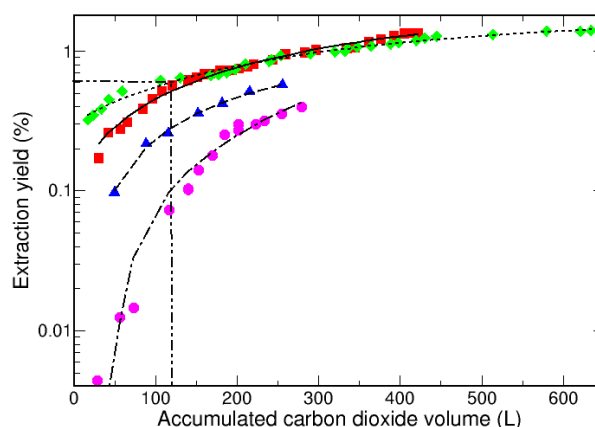

Figure 1. Extraction yield as function of CO2 consumed (calculated as $accumulated\ CO_2\ flowrate\ multiplied\ by\ time$) for E1 (magenta circles) at 320 bars and 90°C; E2 (red squares) at 640 bars and 90°C; E3 (green diamonds) at 640 bars and 40°C; E4 (blue triangles) at 320 bars, 90°C and 2% co-solvent.

When studying the influence of the fluid density on the extraction, the pressure has a more marked effect than temperature. In fact, while pressure usually can vary up to 8 or 10 times the critical values (ca. 73 bar), the temperature is restricted to a narrower variation window, i.e. only up to an increase of 30% the critical temperature of 31◦C [3].

Figure 2, presents pressure and temperature profiles for extraction. The process begins with a preparation time (approximately 100 minutes) in which the pressure and temperature for the extraction are adjusted. The figure shows that at the end of this stage, there is a peak in the profiles, which can be explained in that pressure and temperature are directly proportional and their adjustment is difficult when manipulating them simultaneously. Then, extraction occurs, and values of pressure and temperature keep stable (as shows standard deviation in **Table 2**), Finally, the cleaning step, which consists of cooling the recipe and letting out the pressure, injecting ethanol, and purging with CO_2 at a lower pressure (hence the last slope change in the graph). This step largely depends on out flow rate, a big flow rate can make slope steeper because pressure is letting out faster.

Table 2. Mean and standard deviation for pressures, temperatures and flows during E1, E2, E3, E4 and E5.

	P E1	T E1	P E2	T E2	P E3	T E3	P E4	T E4	P E5	T E5	Flow E1	Flow E5
Mean	323.36	79.90	641.68	85.74	642.15	42.27	339.56	83.53	203.23	28.06	2.71	2.39
St. Dev.	12.00	6.25	4.12	1.02	6.73	0.33	2.60	0.20	5.27	0.43	2.32	0.65

Due to the large size of the extraction vessel high pressures translates into large preparation and cleaning times as shown in **Figure 2**. Comparing E1 and E2, when duplicating the pressure, the preparation time increases 1.72 times.
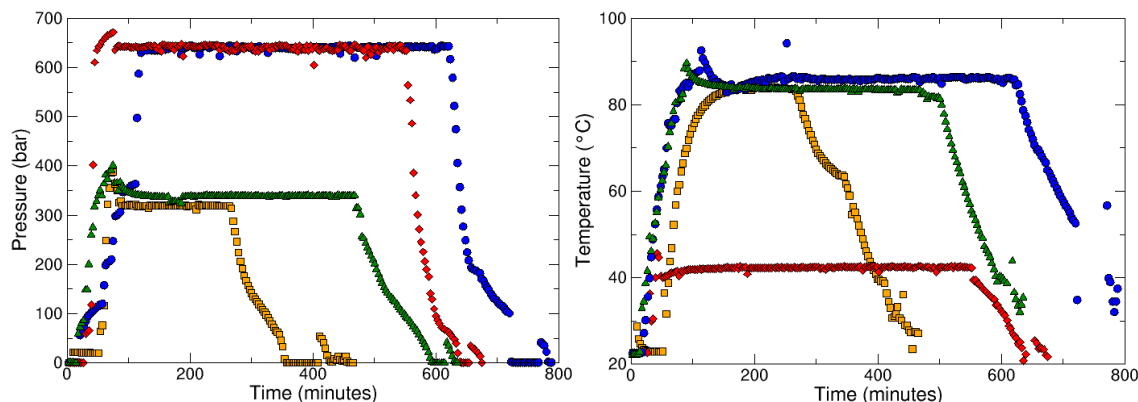

Figure 2. *Pressure and temperature extraction vessel profiles for E1 (orange square), E2 (turquoise circles), E3 (red diamonds), E4 (green triangles)*

High pressure can also be reflected in a larger CO_2 consumption, 1.56 kg were calculated for extraction at higher pressure and temperature, without considering the cleaning process (this corresponds to 6.24% of the total content of the CO_2 cylinder). Therefore, it is proposed to: (i) adapt the connections of the system for the smaller size vessel (300 ml) available as separator, (ii) the recirculation of the CO_2 (at least for use in cleaning) and/or (iii) cleaning the system after the extraction using compressed air instead of CO_2.

In addition, during the operation at high pressure, it was found that after extraction the stevia matrix was charged electrostatically. This could be due to the glass wool being statically charged, due to high molecular activity [36].

Performing the extraction with the 300 ml separation module allowed more stability for pressure and flowrate on the system. In **Table 2**, standard deviation is 3.6 times smaller for E5 flow than E1 flow, and deviation in pressure is 2.3 times smaller.

SFE has distinctive advantages for on-line fractionation, as it allows the extraction conditions to be fine-tuned with a view to improving specific extractions. Among other things, this allows one to separate extracted compounds into groups by adjusting operational parameters, such as the pressure and/or temperature of the extraction [35]. Separator vessel use creates an opportunity for product fractionation. **Figure 3** shows the Infrared spectra for the extracts obtained with the extraction module connected at 30 bars and 35°C. There is not a difference between the peaks in the figure, it means, samples present same functional groups, so it is concluded that fractionation requires a deeper experimental evaluation to determine what conditions allow to achieve separations of different compounds on the same extraction.
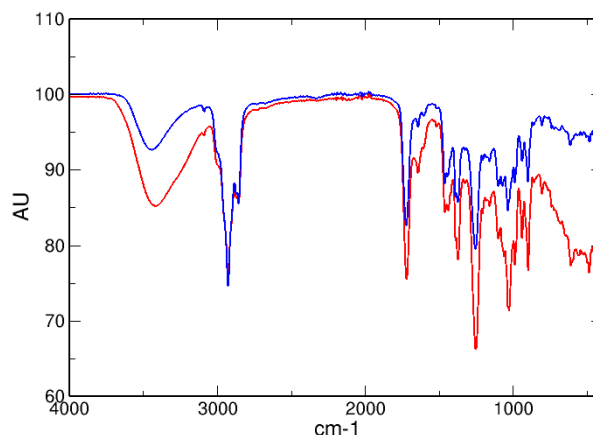
Figure 3. *IR spectrums for extracts obtained using separator vessel connected to system. Separator fraction in blue and collector fraction in red.*

From **Figure 1** an approximate extraction time of 120 minutes was determined for extractions.

The influence of the solvent flowrate was evaluated in relation to the response of the equipment rather than in terms of yield. It was found that high CO_2 flows (above 6 L/min) can cause material losses by dragging extract through the outlet gas. Also, high flowrate can cause extract freezing due to Joule-Thomson cooling plugging the pipes. Although, the mass transfer increases with the decrease in flow rate because contact time between solvent and compounds increase [37], the velocity of solvent can be incremented to enhance extraction by reduction film resistance to mass transfer [3]. For preliminary extractions performed at 200 bars, 60°C and 105 minutes, a yield of **0.21%** was obtained for a CO_2 flowrate of 0.9 L/min, and **0.26%** for 3.9 L/min flowrate.

The effects of modifying the matrix can be seen in **Table 3**. The final yield obtained is very similar for both matrixes, although the extraction conditions reported in literature for supercritical fluid extraction was different for each plant. Hinojosa et al [22] reported 400 bars, 75°C and 20% co-solvent for glycosides from Stevia extraction. For Gliricidia Sepium, literature reported values of 40°C and 220 bars [38]; 40-60°C and 100 to 300 bar [39]; 40°C, 300 bar and 40 minutes of extraction[40]. The extractions were performed at conditions nearer to those reported for Gliricidia. This suggest that conditions favor extraction of Gliricidia rather than Stevia, this is reflected in a smaller yield obtained for stevia.

Table 3. *Particle size, consumed CO2 and yield for both matrixes tested at 200 bars, 40°C and 130 minutes of extraction*

Matrix	Particle size (cm)	Consumed CO2 (L)	Yield (%)
Gliricidia Sepium	0.06	290.94	0.475
Stevia Rebaudiana	0.8	370.99	0.397

In **Table 3** particle size effect can also be observed, it is suggested than yield obtained was better for Gliricidia Sepium, the plant with small particle size (0.06 cm). Matrix size reduction process is important to increase the mass transfer; however, the experiments are not comparable because the affinity of each matrix with the solvent is different.

The mass transfer increases with the increase in the surface area of the matrix, however a small size can difficult matrix manipulation: also, dragging of material can occur plugging the container bag, the tank outlet filter and even the pipes and valves.
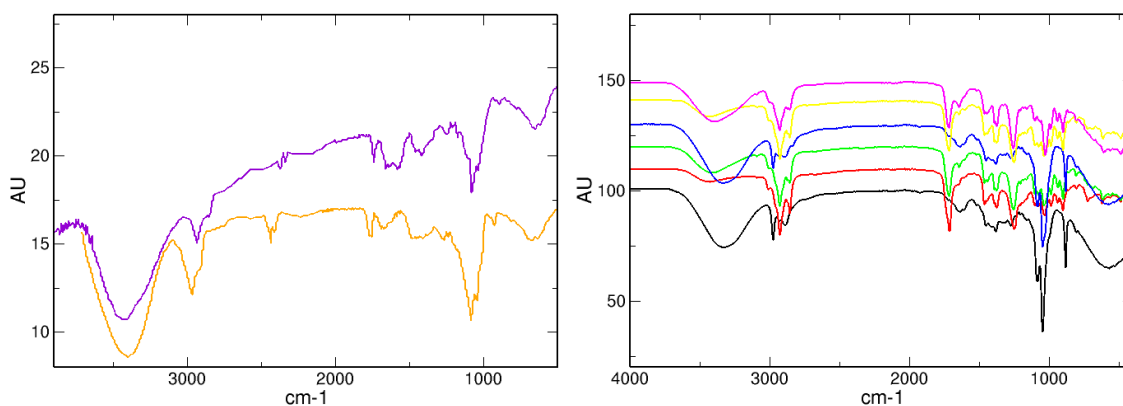

Figure 4. *Right: Infrared spectra obtained for ES (black line), E1 (red line), E2 (green line), E3 (blue line), E4 (yellow line), E6 (magenta line) and Left: IR spectra for standard of rebaudioside A (orange line) and stevioside (violet line)*

According to the infrared spectra obtained (**Figure 4**), the presence of glycosides in the samples is observed, however, due to the similarity of the compounds, it is not possible to determine, from this test, which type of glycoside was obtained. The samples present similar spectra, expect Soxhlet extraction, that shows a missing peak at 1700-1800 cm-1, that can correspond to a double bond between carbons. This suggests that CO_2 extracted a compound that ethanol could not.

HPLC results are shown on **Figure 5**. The obtained stevia extracts present several peaks between 2 and 8 minutes, indicating the presence of several compounds in the samples Comparing the retention time of the two standards (2,007 min for stevioside and 2,014 min for rebaudioside A), it is difficult to determine the amount of these glycosides in the sample obtained because it is not possible to differentiate them. However, in the samples, the most pronounced peaks are found around 2 minutes assuring the presence of at least one of the glycosides in the extracts. Soxhlet extraction presents the major amount of the glycosides, as the height of the peak shows.

For E4, absorbance values are under zero after 7 minutes, which can be explained in that the equipment was not stable. For E2, E6 and Soxhlet extraction, it is hard to differentiate peaks, this could occur because sample was too concentrated, and separation was not achieved and HPLC conditions. This kind of test use to be performed with a gradient in mobile phase that can help to separation.
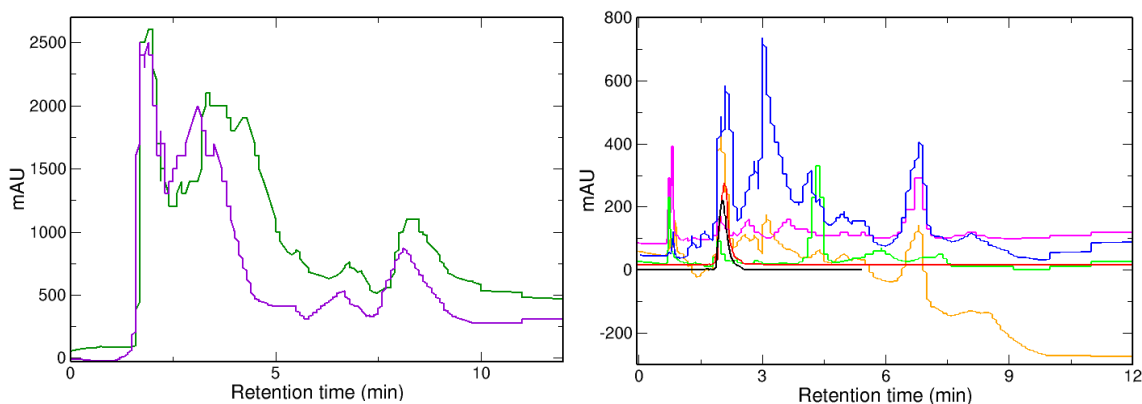

Figure 5. *Chromatograms for obtained extracts. Rebaudioside A standard (black line), Stevioside standard (red line), E1 (green line), E2 (blue line), E4 (orange line), E6 (magenta line). Soxhlet extraction (dark green line) and E3 (violet line)*

4. Conclusions and recommendations

The effect of different extraction variables was evaluated for the EAFIT's Helix Supercritical Fluid System by extraction of a natural product. Pressure seems to be the most influential variable in capability of Helix System, both extraction yield and performance.

The extraction yield as a function of the volume of CO_2 consumed is a good indicator of the kinetic effects of the controlled variables for the Helix supercritical fluid equipment.

The maximum yield (1.4%) was obtained at a pressure of 640 bar, 90 °C and an extraction time of 363 minutes. Due to the low yield obtained in contrast to the value obtained by Hinojosa et al. [22] (2.89% for a 45 minutes extraction at 400 bars, 75°C and 20% co-solvent) and the value obtained by ethanol Soxhlet extraction (3.67%), it is suggested that extraction with supercritical carbon dioxide is not efficient in terms of yield for obtaining stevia glycosides.

The pressure was the variable with the largest influence on the extraction, while the temperature did not affect the yield. Similarly, the use of ethanol as a cosolvent remarkably improved the performance at same conditions of pressure and temperature. A more though study is required to evaluate the influence of the amount of ethanol of it in the extraction yield.

IR and HPLC confirmed that the obtained extracts do contain the expected Stevia glycosides, although the amount was not determined due to the difficulty of differentiation between rebaudioside A and stevioside retention times and spectra. In addition, the existence of other peaks in HPLC analysis suggests the presence of other compounds in the extracts.

An operation manual for the Helix supercritical extraction equipment was written, this represents an important tool for performing extractions that allow creating value added natural products. Further studies in the use of the equipment are recommended for a better understanding of the extraction technique and the use of advanced separation techniques, such as SFE, by process engineering students.

5. Table of annexes or appendices

Table 4. Additional documents

Name	Development	Type of file	Link
Manual del Sistema de Fluidos Supercríticos Helix	Own	pdf	http://xurl.es/nlpaz
Consumo de CO_2	Own	xslx	http://xurl.es/5ebq7

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