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RESEARCH ARTICLE

# Construction and Validation of an Online Subcritical Adsorption-based Device for Assisting $CO_2$ Recycling during a Supercritical Fluid Extraction Process

R. Abel C. Torres, Diego T. Santos and M. Angela A. Meireles*

*LASEFI/DEA/FEA School of Food Engineering/UNICAMP University of Campinas, R. Monteiro Lobato, 80; 13083-862, Campinas, SP, Brazil*

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**Abstract:**

***Background:***

An efficient process for extracting food ingredients from plant materials should demand the use of a reduced volume of extraction solvent to obtain a final product that is free of solvent and reduces both the processing time and the costs. In some cases, achieving a new efficient process requires the modification, reconfiguration or renewal of elements that are part of a processing unit.

***Objective:***

The goal of this work is to describe the development of a modification of a commercial supercritical fluid extraction pilot unit designed to assist $CO_2$ recycling based on subcritical adsorption on an adsorbent material. In addition to the construction and validation of the system, a cost survey was performed to estimate the cost of the homemade device developed to allow effective $CO_2$ recycling.

***Methods:***

The developed device was tested using cotton and Celite® as model adsorption materials and annatto seeds (*Bixa orellana L.*) as a model plant material. A 0.65 L adsorption column was installed with a set of connections and valves that complemented the unit's recycle system. The validation procedure consisted of defatting annatto seeds.

***Results:***

The proposed online subcritical adsorption-based device was technically validated using cotton as an adsorbent material. The cost survey showed that an estimated total cost of USD 5731.36 would be required to install the developed device in a Supercritical Fluid Extraction (SFE) unit similar to the one coupled here (5 L).

***Conclusion:***

The proposed device was demonstrated to be very promising for application in the pseudocontinuous SFE, recirculating the same amount of $CO_2$ mass exceeding the S/F values by 14 times, when compared to a process without a $CO_2$ recycling system.

**Keywords:** *Bixa orellana L*, $CO_2$ recycle, Online coupling, Plant extracts, SFE, Supercritical $CO_2$.

## 1. INTRODUCTION

Several substances that can confer or intensify color, such as carotenoids (β-carotene, lycopene, lutein and zeaxan-

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* Address correspondence to this author at the LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), CEP 13083-862, Campinas, SP, Brazil; Tel: +55 19 35210100; E-mail: maameireles@lasefi.com

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thin) [1, 2], are added to foods to confer an extensive range of colors, including shades of red, orange and yellow [3]. There is a growing interest in using natural pigments because they present a wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer activity [4 - 6].

Annatto seeds contain several carotenoid derivatives (including bixin and norbixin), terpenoids, tocotrienols, aromatic hydrocarbons, and flavonoids [7]. According to Rosa *et al.* [8], annatto seed oil is rich in tocotrienols, especially γ- and δ-tocotrienols.

To obtain these compounds from annatto seeds, some conventional extraction techniques have been studied, such as extraction in oil, alkaline aqueous solutions and organic solvents [9] and mechanical extraction [10].

However, applications whose processes are called clean extraction technologies have also been studied. For example, Supercritical Fluid Extraction (SFE) has been studied by Albuquerque & Meireles [11], Moraes *et al.* [12] and Johner & Meireles [13]; pressurized liquid extraction (PLE) has been studied by Rodrigues *et al.* [14]; and Supercritical Fluid Extraction of Emulsions (SFEE) and Supercritical Antisolvent Precipitation (SAS) have been studied by Mattea *et al.* [15] and Santos & Meireles [16], respectively.

Based on clean extraction technologies, an efficient process for extracting food ingredients from plant materials should require the use of a small volume of extraction solvent to obtain the product without the presence of the solvent in the extract, reducing both the processing time and production costs. At the same time, efficient extraction contributes to the reduction of $CO_2$ emissions, which represents an international concern [17], as well as the search to reduce $CO_2$ emissions in relation to the energy generated to achieve such a reduction [18]. In Brazil and Latin America, the extraction of bioactive compounds from vegetable matrices using $CO_2$ as a solvent represents an attractive field [12]. As far as is known, semi-industrial commercial units are provided with a recycling mechanism for $CO_2$ that is sometimes reported to have some limitations in terms of functionality, since part of the extract goes to the reservoir of the recycling system, which limits its permanent use. One reason for this limitation is the high $CO_2$ flow rate that causes an inefficient separation. Decreasing $CO_2$ flow rate can be a possible solution. However, it consequently increases the extraction duration. Another possible solution is employing an adsorption column. Achieving new and efficient adsorption processes requires, in some cases, the modification, reconfiguration or renewal of the elements that are part of the processing unit. Therefore, the present work is related to these adaptations and details the modification of a commercial supercritical fluid extraction pilot unit to assist $CO_2$ recycling based on subcritical adsorption on an adsorbent material. Cotton and Celite® 512 medium were used as model adsorption materials, and annatto seeds (*Bixa orellana L.*) were used as a model plant material. In this context, this study aims to describe the development of a modification of a commercial supercritical fluid extraction pilot unit designed to assist $CO_2$ recycling based on subcritical adsorption on an adsorbent material. In addition to process construction and validation, a cost survey was performed to estimate the cost of the homemade device developed to allow effective $CO_2$ recycling.

## 2. MATERIALS AND METHODS

### 2.1. Raw Material Characterization

Annatto seeds of the variety Piave (*Bixa orellana L.*) were donated by Urucum do Brasil Ltda, located in Santa Marta, Monte Castelo - SP, Brazil. The seeds were stored at 291 K in a freezer (VF55D, Metalfrio, São Paulo, Brazil). The mean diameter of the annatto seeds, 3.82 ± 0.09 mm, was calculated by an adaptation of Silva *et al.* [19]. A total of 30 annatto seeds were removed from 3.5 kg of seeds for the measurement of the lengths of their axes. Then, to repeat the measurement of lengths, 30 seeds were placed back into the bag (which again weighed 3.5 kg), and after homogenization, the procedure was repeated.

The moisture content of the annatto seeds, 11.61 ± 0.02 g/100 g, was analyzed as described by AOAC [20] and was determined in triplicate by drying at 378 K until reaching constant mass. The true density (1.35 ± 0.01 g/cm$^3$) of the particles was measured by pycnometry with helium gas at the Central Analytical Laboratory of the Institute of Chemistry - UNICAMP (Campinas, Brazil). The apparent density (0.7609 ± 0.0000 g/cm$^3$) in SFE bench-scale unit, of the bed was calculated by dividing the sample feed mass by the extractor volume. The porosity of the bed was calculated by equation 1.

$$\varepsilon = 1 - \frac{\rho_a}{\rho_r} \tag{1}$$

where $\rho_r$ is the true density of the particles; $\rho_a$ is the apparent density of the bed; and $\varepsilon$: is the porosity of the bed.

### 2.2. Description of Supercritical Fluid Extraction Units

Two commercial supercritical fluid extraction units were used in this study, a bench-scale unit and a pilot-scale unit. In the bench-scale unit, the extraction kinetics of annatto seeds was investigated. However, in the pilot-scale unit, several elements were changed and adapted to test the possibility of recycling $CO_2$ without creating drag or with the least amount of drag, which would prevent the $CO_2$ from entering the extraction column.

#### *2.2.1. SFE Bench-scale Unit*

The main elements that compose the commercial SFE bench-scale unit (Spe-ed model, Applied Separations, Allentown, USA) are represented in Fig. (**1**). The extraction column used in this study has a total volume of 25 mL, height of 83.5 mm, and diameter of 20.0 mm.

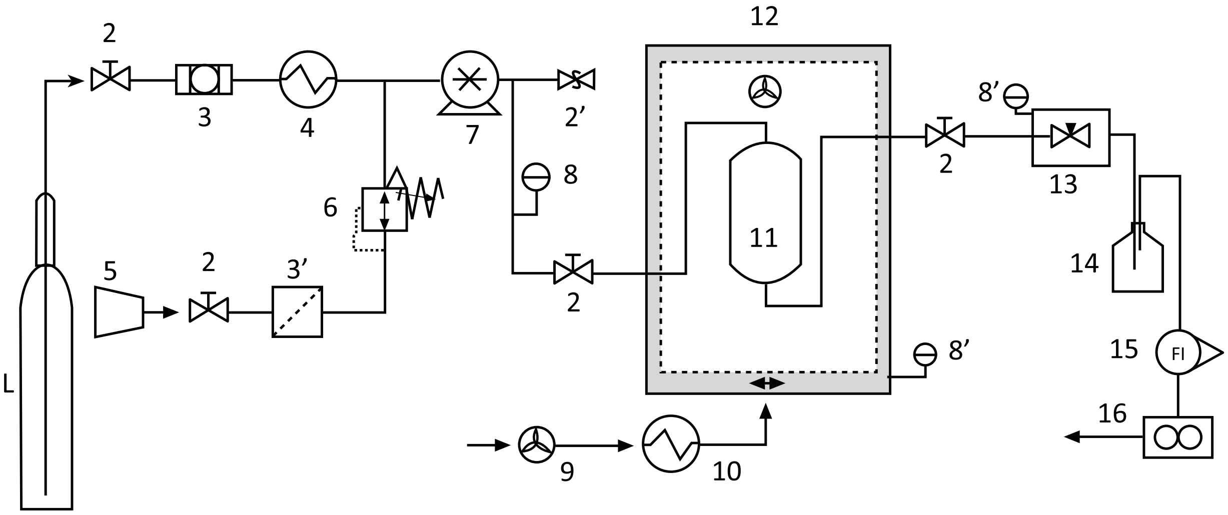

**Fig. (1).** SFE bench-scale unit. (1) $CO_2$ cylinder, (2) blocking valve, (2') safety valve, (3) line filter, (3') air filter, (4) cooling bath, (5) compressor, (6) air pressure regulator, (7) air-driven fluid pump, (8) manometer, (8') temperature indicator, (9) blow motor, (10) blow motor resistor, (11) extraction column, (12) oven module, (13) micrometric valve with heating system, (14) collection flask, (15) flow meter, (16) flow totalizer.

The operational sequence was as follows: $CO_2$ was cooled at 263 K by a cooling bath (Logen Scientific, LS-540, Diadema, Brazil) before reaching the pump. The desired temperature for $CO_2$ was obtained using hot air circulation by blow motor and blow motor resistor inside the oven module, which included the extraction column and the pipes connected to the inlet and outlet of the column. Accordingly, $CO_2$ transited a thermodynamic phase change from liquid state to supercritical state. Then, the extractor was kept at 20 MPa and 313 K for 20 min (static time) to get saturation of the solvent in the extractor before solvent pumping. After that, the outlet valve of extractor was opened, and the dynamic time of extraction was started. The extract was collected in a 100 mL glass vial immersed in an ice bath at ambient pressure. The total $CO_2$ was measured with a flow totalizer (Itrón, model G1.0, Campinas, Brazil), and the mass flow for each process was calculated in an Excel spreadsheet, weighting the values of the density of the $CO_2$ found elsewhere [21] keeping the environment at a constant temperature; $CO_2$ was not recirculated. After the extraction, the glass vial was placed in a desiccator for thirty minutes to guarantee the complete elimination of $CO_2$.

#### *2.2.2. SFE Pilot-scale Unit Description*

The commercial SFE pilot-scale unit (Thar Technologies, model SFE-2X5 LF-2-FMC, Pittsburgh, USA) used in this study was acquired in the framework of the PITE/FAPESP project (05/60948-1) in partnership with the company CENTROFLORA (Botucatu, SP) during the period 2006-2009.

The main elements that compose the initial state of the commercial SFE pilot-scale unit are represented in Fig. (**2**). The extraction column used in this study has a total volume of 5 L, height of 620 mm, and diameter of 106 mm. The open-closure sealing system is an internal thread with a depth of 7 threads x 39.0 mm and the cover is the external thread with a depth of 6 threads x 36.0 mm and sealing gasket (the maximum operating pressure and temperature are 68.95 MPa and 363 K, respectively).

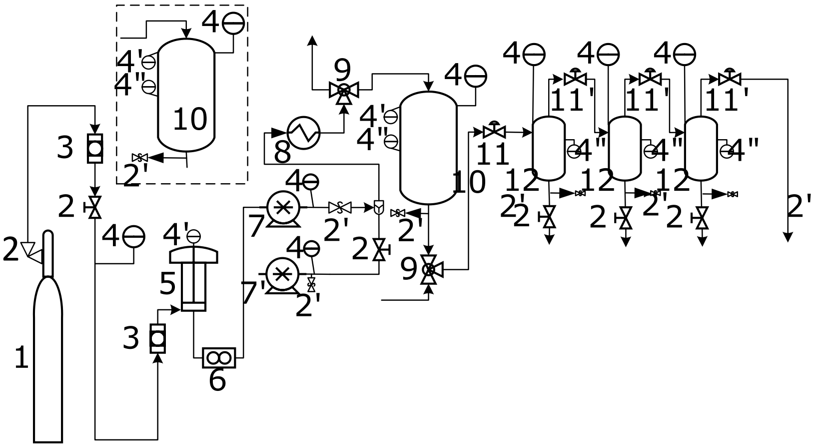

**Fig. (2).** Initial state of SFE pilot-scale unit. (1) $CO_2$ cylinder, (2) blocking valve, (2') safety valve, (3) line filter, (4) manometer, (4') internal temperature indicator, (4") external temperature indicator, (5) heat exchanger with coiled tube for $CO_2$ cooling, (6) mass flow meter, (7) $CO_2$ pump, (7') cosolvent pump, (8) heat exchanger for $CO_2$ heating, (9) three-way valve, (10) extraction column, (11) automated backpressure valve, (11') manual backpressure valve, (12) separation vessel.

The operational sequence was as follows: $CO_2$ passed through the heat exchanger with coiled tube where it was cooled at 263 K by a cooling bath (Polyscience recirculator 6100T, Niles, Illinois, USA) and for the mass flow meter (Siemens Coriolis flowmeters SITRANS F C MASS 2100 Di 1.5), before reaching the pump (maximum flowrate of 350 mL/min, maximum operating pressure of 34.47 MPa). Then $CO_2$ passed through a heat exchanger, which raised the temperature and entered into the extraction column equipped with an electric heating jacket (Ogden Mighty-Miser CBE06A12A-02212, 2000 W, 240 V) kept to 313 K and 20 MPa for 20 min (static time) to get saturation of the solvent in the extractor before solvent pumping. After that, outlet three-way valve of extractor and the automated backpressure valve were opened, and the dynamic time of extraction was started. The extract was collected in three separators (Cyclone type, volume of 1 L, diameter of 74 mm, height of 275 mm. Each one with an electric heating jacket, Ogden Mighty-Miser CBE04J04A-02158, 500 W, 240 V). The total $CO_2$ was measured with a flow totalizer (Itrón, model G1.6, Campinas, Brazil), and the mass flow for each process was calculated in an Excel spreadsheet, weighting the values of the density of the $CO_2$ found elsewhere [21] keeping the environment at a constant temperature. $CO_2$ was recirculated in 4 of 5 experiments (B, C, D and E). After the extraction, the extract was collected in glass bottles of 100 mL and placed in a desiccator for thirty minutes to guarantee the complete elimination of $CO_2$.

### 2.3. Supercritical Fluid Extraction Experiments

#### *2.3.1. SFE Bench-scale Unit Experiments*

$CO_2$ was used as the solvent (99.9% $CO_2$, Gama Gases Especiais Ltd, São Bernardo do Campo, Brazil) The global extraction yield (X0) was obtained from kinetic curve data corresponding to the diffusion-controlled period [22]. The selected extraction conditions used in this study (313 K and 20 MPa) were set based on a previously optimized method for the recovery of tocotrienol-rich oil from annatto seeds by SFE developed by Albuquerque & Meireles [11] in an SFE bench-scale unit (290 mL). A 25 mL extractor was filled thoroughly with annatto seeds (15.4689 ± 0.0001 g (d.b.), the $CO_2$ flow rate (11.10 ± 0.04 g/min) was kept constant. After that, the extraction bed was filled, and a plug of glass wool was placed on both the lower and upper ends of the extraction column. The relative yield (R, g extract/100 g of extractable) for the extraction was calculated using equation 2:

$$R\left(\frac{g\ extract}{100\ g\ of\ extractable}\right)=\frac{mass_{extract}\left(\frac{S}{F}\right)}{mass_{extract}\left(\frac{S}{F}=259\right)}x\ 100 \tag{2}$$

In our study, the global extraction yield (X0) was determined by the summation of the extract obtained from ethanol cleaning (after evaporation of the solvent) [23] and the extract obtained during the extraction kinetics experiment. Then, the glass vials were stored at 255 K until analysis. The X0 data obtained in this unit were compared with the data obtained in the pilot-scale unit.

The experimental overall extraction curve (OEC) data obtained using the bench unit were adjusted to fit a spline with three straight lines using the software SAS 9.2®. This software allowed the annatto oil extraction kinetic parameters to be determined, including the constant extraction rate (CER) period ($t_{CER}$), the mass transfer rate for the CER period ($M_{CER}$), the yield for the CER period ($R_{CER}$), the mass ratio of solute in the fluid phase at the extractor outlet for the CER period ($Y_{CER}$), the falling extraction rate (FER) period ($t_{FER}$), the mass transfer rate for the FER period ($M_{FER}$), the yield for the FER period ($R_{FER}$) and the mass ratio of solute in the fluid phase at the extractor outlet for the FER period ($Y_{FER}$).

Jesus *et al.* [24] describe the spline model adopted in this study with three periods using extraction kinetics data:

$$\begin{gathered}when\ t \leq t_{CER}: \\ m_{EXT}=b_0+a_1 t\end{gathered} \tag{3}$$

$$\begin{gathered}when\ t_{CER} < t \leq t_{FER}: \\ m_{EXT}=b_0+a_1 t+a_2(t-t_{CER})\end{gathered} \tag{4}$$

$$\begin{gathered}when\ t > t_{FER}: \\ m_{EXT}=b_0+a_1 t+a_2(t-t_{CER})+a_3(t-t_{FER})\end{gathered} \tag{5}$$

where $t$ is the time of extraction (min); $m_{EXT}$ is the mass of extract (g); $b_0$ is the linear coefficient (zero-order term) of the CER straight line (g); and $a_1$, $a_2$ and $a_3$ are the slope coefficients (first-order terms) of the CER, FER, and DC (diffusion-controlled) straight lines, respectively (g/min).

According to the results of Meireles [25] and Moraes *et al.* [26], $t_{CER}$ is the intersection of the first straight line with the second, and $t_{FER}$ is the intersection of the second straight line with the third (DC, diffusion-controlled) straight line.

Noticeably, this model is exclusively used for extractor, and it does not cover the adsorption column.

### *2.3.2. SFE Pilot-scale Unit Experiments*

The extraction conditions (313 K and 20 MPa) set for the bench-scale unit for defatting annatto seeds were set for the SFE pilot-scale unit for 5 experimental runs. Based on previous studies performed in a pilot-scale unit, the automated backpressure valve was opened to flow $CO_2$ with the mass flow rate of 200 g/min [23]. The fractionation of the extracts by pressure and temperature reduction was also performed to obtain the heaviest fraction in the first separation vessel (SV), and the lighter fractions in the following separator vessels were as follows: SV1 ($P = 8.0$ MPa, $T = 313$ K), SV2 ($P = 6.5$ MPa, $T = 303$ K) and SV3 ($P = 6.5$ MPa, $T = 313$ K). These data were defined through detailed literature review. The use of a higher pressure reduces the cost of recompressing the solvent for recycling within the process [27]. Thus, Prado [23] inferred that a pressure of approximately 6 MPa may be economically interesting for use in separator vessels at an industrial level. Therefore, in this study, we chose to set the pressure of the last separator vessel (SV3) at 6.5 MPa.

There were 5 experimental runs carried out in the SFE pilot-scale unit. Experimental run A was developed according to the initial state just as the pilot-scale unit was found (in other words without any modification). The recycling and adsorption system was adapted to the pilot-scale unit to carry out the experimental runs from B to E. In the experimental runs, B and C, and, D and E, silicon dioxide (Celite® 512 medium) and cotton (in a uniform orthopedic blanket roll) (Hydrophilic Cotton, Medi House, São Paulo, Brazil), respectively, were tested as adsorbent materials for the validation of the developed online subcritical adsorption-based device for assisting $CO_2$ recycling during SFE. When using the Celite® 512 medium, the 0.65 L adsorption column was filled with 150 g of silicon dioxide inserted to the bottom part of the AdC, and a plain weave stainless steel disc filter (wire diameter 0.18 mm, aperture size 0.15 mm) was inserted on the top of silicon dioxide. The remaining part of AdC was filled with 0.43 kg of glass beads with a diameter of 0.3 mm, but when using cotton, only 100 g filled the adsorption column.

The tendency of the effect of three moments of pressurization/depressurization was studied in the experimental runs B and C, in that a single moment of pressurization/depressurization was applied for the experimental runs D and E.

### 2.4. Description of SFE Pilot-scale Modifications

To recirculate $CO_2$ at purity conditions similar to those of the initial feed, an AdC was installed between the SV3 outlet and the $CO_2$ condenser, which was connected to what is here called the lung tank (LT). The capacity of LT is 15 L volume, having a maximum allowable working pressure of 10.34 MPa at 298 K, and it is made of 304 stainless steel.

Fig. (**3**) shows the initial step of the recycle system construction, as well as its main components.

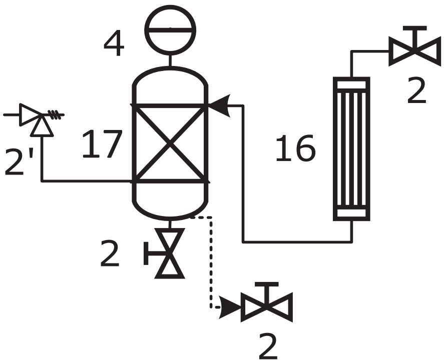

**Fig. (3).** Initial step of the recycle system construction. (2) blocking valve, (2') safety valve, (4) manometer, (16) shell and tube heat exchanger, (17) lung tank (LT).

The purpose of installing an AdC (this column was maintained at an average room temperature of 295 K, it has no heating system) as part of the initial $CO_2$ recycle system was to keep the $CO_2$ mass in the same condition as if it were entering the system for the first time (*i.e.*, 99.9% purity). To this end, a 0.65 L (height of 22 cm and diameter of 12 cm) AdC was installed with a set of connections and valves that complemented the pilot-scale unit's recycle system. Fig. (**4**) shows the sequence of connections, valves, and piping that was installed in the final stage of the study, and Fig. (**5**) shows the final state of the SFE pilot-scale unit after the inclusion of the online subcritical adsorption-based device (shown in red).

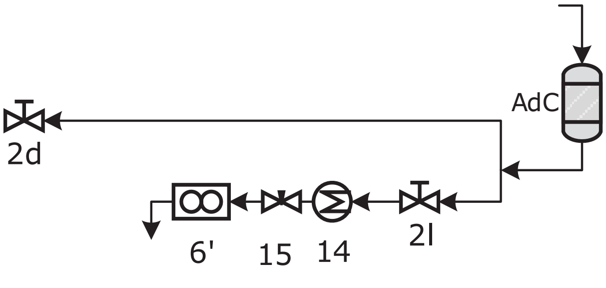

**Fig. (4).** Final step of the recycle system construction. Adsorption column (AdC) and other components as part of the recycle system of the pilot-scale unit. (2) blocking valve, (6) diaphragm flow meter, (AdC) adsorption column, (14) heat exchanger with electric resistance to heat the micrometering valve, (15) micrometering valve.

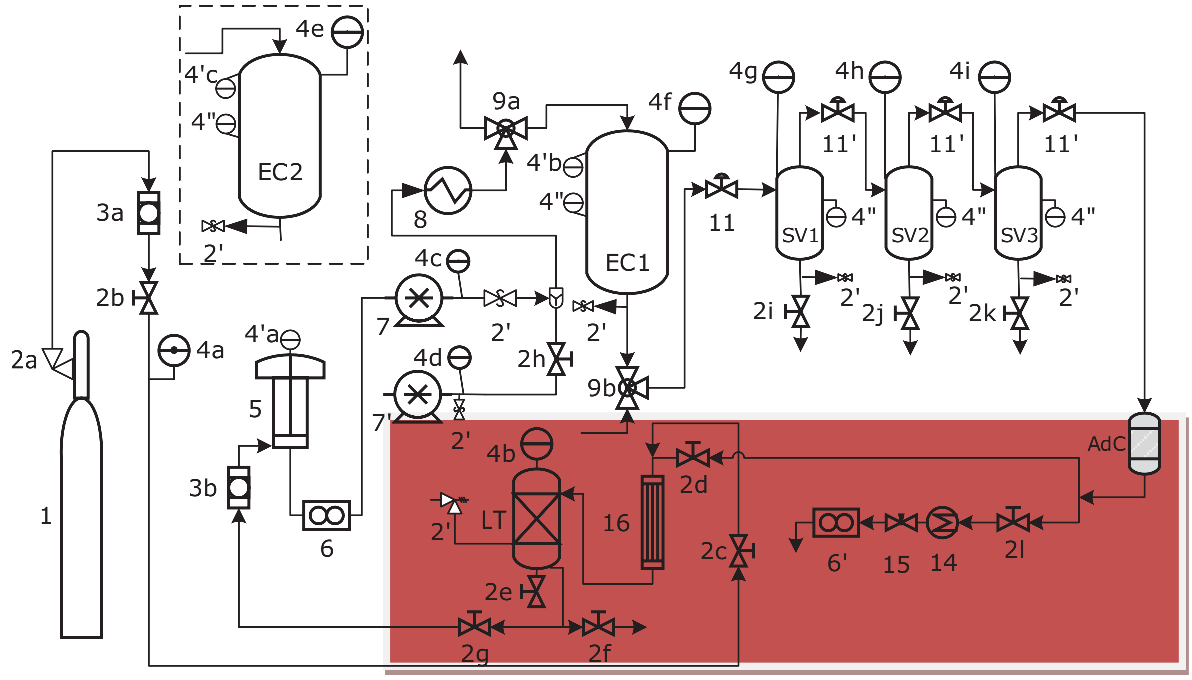

**Fig. (5).** Final state of SFE pilot-scale unit after online subcritical adsorption-based device inclusion. (1) $CO_2$ cylinder, (2) blocking valve, (2') safety valve, (3) line filter, (4) manometer, (4') internal temperature indicator, (4") external temperature indicator, (5) heat exchanger with coiled tube for $CO_2$ cooling, (6) mass flow meter, (7) $CO_2$ pump, (7') cosolvent pump, (8) heat exchanger for $CO_2$ heating, (9) three-way valve, (10) extraction column, (11) automated backpressure valve, (11') manual backpressure valve, (12) separation vessel, (13) adsorption column (AdC), (14) heat exchanger with electric resistance, (15) micrometering valve, (16) shell and tube condenser, (17) lung tank (LT).

To facilitate cleaning and to understand in detail the parts that composed the developed recycle device, all the connections were disassembled to evaluate the possibility of avoiding dragging the extract toward the LT (17, in Fig. (**3**)). The blocking valve located at the inlet of the refrigeration heat exchanger ($CO_2$ condensing column) can operate at up to 41.37 MPa CWP (cold working pressure) and 311 K. The shell and tube heat exchanger used for refrigeration was of model # 00256-06 (Garden City, NY, USA), and the tube side pressure could withstand maximum conditions of 8.3 MPa [28]. The shell and tube heat exchanger contains 61 numbers of tubes with length and diameter of 51 cm, and 2.5 mm respectively. The cooling solution, composed of water + ethylene glycol (50:50 v/v), entered the side of the heat exchanger by applying pressure to the inner pipes of the exchanger. According to PolyScience [29], the maximum pressure exerted by the recirculating cooling bath (PolyScience recirculator 6100T - 230 V / 60 Hz / 12.2 A / 1 HP, Niles, Illinois, USA) that drives the refrigerant solution is 0.62 MPa. In addition, the minimum range of the flow rate alarm set point is 3 to 8 LPM (liters per minute), and the maximum flow rate at 0 MPa is 13.2 LPM [30].

With the modifications made in the recycling system, the experiments from B to E were developed as follows: the processes B and C are as follows: 1) The extractor was pressurized from 0.1 MPa to 20 MPa during 20 min. Then, the extractor remains in this condition for 20 min (static time). After that, the extractor was depressurized to 7 MPa. 2) The extractor was pressurized again from 7 MPa to 20 MPa during 10 min and remained in this condition for 20 min (static time). Then, the output valve of extractor was opened to start the dynamic extraction until reaching values of S/F ratio (g $CO_2$/g annatto) 25, for B, and 10.36, for C. Next, the recycling valve (2d in Fig. (**5**)) was closed, and the ambient valve (2l in Fig. (**5**)) was opened to flow out the $CO_2$ to the ambient. After that, the extract was collected and weighted. 3) The same procedure of part 2 was repeated except that the pressurization occurs from 0.1 MPa to 20 MPa during 20 min.

In cases D and E, first the extractor was pressurized from 0.1 MPa to 20 MPa during 20 min. Then, the extractor remains in this condition for 20 min (static time). After that, the output valve of extractor was opened to start the dynamic extraction until reaching values of S/F 25.45, for D, and 55.98, for E. Next, the recycling valve (2d in Fig. (**5**)) was closed, and the ambient valve (2l in Fig. (**5**)) was opened to flow out the $CO_2$ to the ambient.

## 3. RESULTS AND DISCUSSION

### 3.1. Effects of the Ratio of Solvent Mass to Feed Mass on the Extraction Yield Using the SFE Bench-scale Unit

The global extraction yield (X0) was obtained from kinetic curves corresponding to the diffusion-controlled period. In practical aspects, the global extraction yield (X0) represents the maximum amount of extract that can be recovered from a raw material at a given extraction pressure and temperature. In contrast to the bench-scale unit, in the pilot-scale unit, it is not possible to collect extracts continuously during kinetics experiments. It is necessary to interrupt the solvent flow and then depressurize the separators while keeping the extractor pressurized so that the extract can be collected, as is the case with experimental runs B and C. After this procedure, the extraction can be resumed until the next collection point. Interrupting the extraction at each point implies intermediary static periods in the extractor. Thus, in this study, we used the bench-scale unit to determine the overall extraction curve (OEC) and X0 for annatto-seed extraction.

Fig. (**6**) shows the OEC obtained for annatto-seed SFE at the bench scale. The results are expressed in terms of the relative yield (R, g extract/100 g of extractable) of the extraction. The term extractable represents the maximum amount of extract that can be recovered from a raw material at a given extraction pressure and temperature within the diffusion-controlled period (S/F ratio of 259 g $CO_2$/g annatto, which is in a good agreement with the value obtained by Moraes *et al.* [26]). The OEC presents a short CER (constant extraction rate) period of 26.17 min, which accounts for almost 60% of the total extract. A DC (diffusion controlled) period follows, with a rapid decrease in the extraction rate; this is the intermediary FER (falling extraction rate) period. The kinetic parameters calculated from the analysis performed using the software SAS 9.2® are in Table **1**, where $t_{CER}$ is the time of the CER, $M_{CER}$ is the mass-transfer rate and $R_{CER}$ is the yield achieved during this period. The value of $t_{CER}$ roughly represents the minimum time an SFE cycle should last to keep the process economically viable [25]. $Y_{CER}$ is the mass ratio of solute in the supercritical phase at the bed outlet during the CER period.

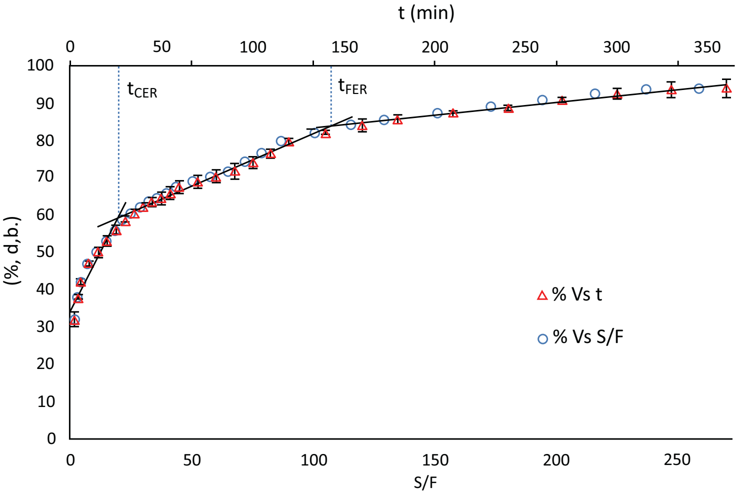

**Fig. (6).** Overall Extraction Curve (OEC) for annatto-seed extraction (313 K and 20 MPa) in the bench-scale unit; (—) indicates data fitted using SAS 9.2®.

**Table 1. Kinetic parameters of SFE from annatto seeds estimated using an SFE bench-scale unit*.**

| b0 ± SD | a1 ± SD | a2 ± SD | a3 ± SD |
|---|---|---|---|
| 33.983 ± 0.578 | 0.997 ± 0.070 | 0.213 ± 0.006 | 0.050 ± 0.026 |
| $t_{CER}$ (min) ± SD | $M_{CER}$ (g/min) ×$10^3$ ± SD | $R_{CER}$ (%) ± SD | $Y_{CER}$ (g ext/g $CO_2$) ×$10^4$ ± SD |
| 24.83 ± 2.94 | 4.34 ± 0.35 | 58.58 ± 2.07 | 8.77 ± 1.09 |
| $t_{FER}$ (min) ± SD | $M_{FER}$ (g/min) ×$10^3$ ± SD | $R_{FER}$ (%) ± SD | $Y_{FER}$ (g ext/g $CO_2$) ×$10^4$ ± SD |
| 138.87 ± 20.98 | 2.27 ± 0.08 | 83.08 ± 3.43 | 2. 23 ± 0.29 |

*The parameters are presented with all digits from SD to avoid rounding errors in case of use of the model for simulating purposes.

Obtaining the $CO_2$ density as a function of ambient pressure (from [21]) together with the monitoring of both ambient pressure (measured through an aneroid barometer) and the volumetric flow of $CO_2$ (measured with the flow totalizer -16 of Fig. (**1**) and with a chronometer), allowed us to control $CO_2$ mass flow rate as accurate as possible. Accordingly, the error for $CO_2$ flow rate, 0.04 g/min, compared to the average of $CO_2$ flow rate, 11.10 g/min, indicates the infinitesimal impact of the precision of $CO_2$ flow rate on both the overall extraction curves and model parameters in this study.

Based on the OEC and kinetic parameters calculated, we also performed 3 runs in the SFE bench-scale system to ensure the reliability of experimental data using different solvent mass to feed mass (S/F) ratios which can be seen in Fig. (**6**). The Table **2** shows the results for the global extraction yield (X0) and residual extract indicators when S/F was varied. It can be observed that both the S/F ratio and the global extraction yield (X0) values had a coefficient of variation close to 1%, which represents a high reliability in the performed process. The Standard deviation reported in this table are related to the uncertainty of the experiments.

**Table 2. Percentage representation of the total extract and the residual extract for defatting annatto seeds using the bench-scale unit.**

| Extraction | Annatto seeds (g) d.b. | S/F | Global extraction yield (X) | | Residual extract indicators | | |
|---|---|---|---|---|---|---|---|
| | | | (g) | % [g extract/ 100 g annatto seeds] | (g) | % [g extract/ 100 g annatto seeds] | % [Percentage of residual extract] |
| 1 | 15.4689 | 257.00 | 0.431 | 2.79 | 0.0382 | 0.25 | 8.23 |
| 2 | 15.4688 | 259.04 | 0.438 | 2.83 | 0.0229 | 0.15 | 5.04 |
| 3 | 15.4690 | 260.02 | 0.440 | 2.84 | 0.0186 | 0.12 | 4.05 |
| Mean ± SD | 15.4689 ± 0.0001 | 259 ± 2 | 0.436 ± 0.005 | 2.82 ± 0.03 | 0.027 ± 0.01 | 0.17 ± 0.07 | 6 ± 2 |
| Coefficient of variation (%) | 0.0006 | 0.60 | 1.040 | 1.04 | 38.7765 | 38.78 | 39.89 |

On the other hand, the process had a high variability with respect to the values obtained related to the extract collected during the cleaning of the unit (residual extract amount), which can be seen in terms of the standard deviation and coefficient of variation, indicating a strong correlation to the S/F ratio. The lower the S/F ratio is, the lower is the X0 and the higher is the accumulation of extract inside the parts of the SFE bench-scale unit, and vice versa. In contrast, approximately 6% of the total amount of extract that can be extracted from the annatto seeds using the SFE conditions used here (313 K and 20 MPa) corresponds to the amount of extract lost from the retention of extract in the pieces of equipment used. The residence time of $CO_2$ in the extractor of bench unit was 0.08 s, while the residence time of pilot unit was from 8.03 to 19.60.

Santos *et al.* [31], using the same commercial equipment, observed a similar percentage of residual extract (7%) for annatto-seed extraction under different SFE conditions (31 MPa, 333 K). The authors also compared commercial equipment with a homemade system that had a similar volume capacity and observed that a significantly higher amount of residual extract was deposited in the tubing line when the homemade equipment was used compared with the amount of residual extract obtained using the commercial SFE apparatus; thus, when using the homemade modification that we applied to the pilot-scale unit, accounting for this loss of extract was a concern.

Before studying the effects of the ratio of solvent mass to feed mass on the extraction yield using the SFE pilot-scale unit, we performed preliminary tests with the developed online subcritical adsorption-based device coupled to the system to validate the device. Silicon dioxide (Celite$^®$ 512 medium) and cotton (in a uniform orthopedic blanket roll) (Hydrophilic Cotton, Medi House, São Paulo, Brazil) were tested as adsorbent materials. The technical viability validation criterion adopted, in addition to visual observation of the adsorbent material after the SFE process, consisted of monitoring the effects of the increased solvent mass to feed mass (S/F) ratio on the extraction yield and percentage of residual extract in the equipment parts and comparing the results for the SFE pilot-scale unit coupled with the online subcritical adsorption-based device with the results for the SFE bench-scale unit.

### 3.2. Preliminary Tests of the Developed Online Subcritical Adsorption-based Device for Assisting $CO_2$ Recycling in an SFE Pilot-scale Unit

Table **3** shows the results for extraction yield and residual extract percentage when solvent mass to feed mass ratio

(S/F) was varied. The term extraction yield is defined as the summation of mass of extract obtained in three separators per 100 g of raw material. Moreover, the term residual extract percentage in this table indicates the amount of residual extract retained inside the tubing system of the SFE unit. Based on this table, the Extraction yield was increased from 2.84, for B, to 3.09, for C. The residual extract was decreased from 8.12, for B, to 6.55, for C. The main reason for this drop of residual extract is the higher value of S/F for C compared to B that leads higher removal of extract available in the tube lines and connections. It can be observed that the values obtained related to the extract collected during the cleaning of the unit (residual extract percentage) presented a strong correlation to the values of S/F ratio.

**Table 3. Percentage representation of the total extract and the residual extract for defatting annatto seeds using a pilot-scale unit with a coupled subcritical adsorption-based device.**

<table>
<tr><th rowspan="2">Run</th><th rowspan="2">Annatto seeds (g) d.b.</th><th rowspan="2" colspan="2">S/F</th><th colspan="6">Extraction yield</th><th colspan="4">Residual extract indicators</th></tr>
<tr><th colspan="2">(g) of extract in the 3 separation vessels</th><th colspan="2">% [g extract/ 100 g annatto seeds]</th><th>(g) of extract/ 100 g of cotton present in the AdC</th><th>% [g extract/ 100 g annatto seeds]</th><th>Separator connections (g)</th><th>Others (g)</th><th>% [g extract/ 100 g annatto seeds]</th><th>% [Percentage of residual extract]</th></tr>
<tr><td>A</td><td>3111.33</td><td colspan="2">3.69</td><td colspan="2">44.31</td><td colspan="2">1.42</td><td>without AdC</td><td>1.42</td><td>6.97</td><td>1.62</td><td>0.28</td><td>16.65</td></tr>
<tr><td rowspan="2">B</td><td rowspan="2">3239.23</td><td>25.00</td><td rowspan="2">44.37</td><td>88.93</td><td rowspan="2">91.98</td><td>2.75</td><td rowspan="2">2.84</td><td rowspan="2">---</td><td rowspan="2">2.84</td><td rowspan="2">7.55</td><td rowspan="2">0.58</td><td rowspan="2">0.25</td><td rowspan="2">8.12</td></tr>
<tr><td>19.38</td><td>3.05</td><td>0.09</td></tr>
<tr><td rowspan="2">C</td><td rowspan="2">3072.43</td><td>10.36</td><td rowspan="2">55.73</td><td>51.79</td><td rowspan="2">94.16</td><td>1.69</td><td rowspan="2">3.06</td><td rowspan="2">---</td><td rowspan="2">3.09</td><td rowspan="2">6.28</td><td rowspan="2">0.32</td><td rowspan="2">0.21</td><td rowspan="2">6.55</td></tr>
<tr><td>45.73</td><td>42.37</td><td>1.38</td></tr>
<tr><td>D</td><td>1326.66</td><td colspan="2">25.45</td><td colspan="2">24.14</td><td colspan="2">1.82</td><td>1</td><td>1.89</td><td>2.85</td><td>0.17</td><td>0.23</td><td>10.73</td></tr>
<tr><td>E</td><td>1326.44</td><td colspan="2">55.98</td><td colspan="2">32.04</td><td colspan="2">2.42</td><td>0.66</td><td>2.46</td><td>2.48</td><td>0.20</td><td>0.20</td><td>7.58</td></tr>
</table>

The higher the S/F ratio, the higher the extraction yield (measured by collecting the extracts in the 3 separation vessels), and the lower the accumulation of extract inside the parts of the SFE pilot scale unit (Residual Extract Percentage %). So, the use of silicon dioxide as adsorbent material for increasing extract recovery besides allow $CO_2$ recycling is an important issue to be addressed.

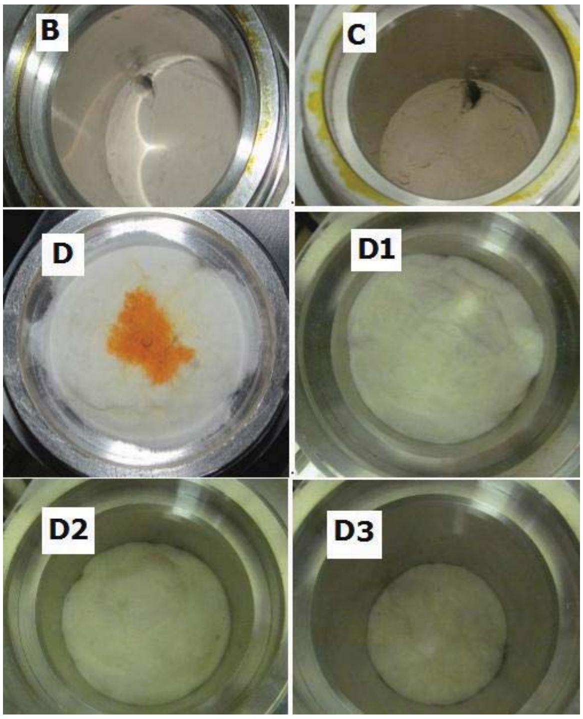

**Fig. (7).** Adsorbent materials used in the online subcritical adsorption-based device. B and C are Celite, and D is cotton. D1, D2 and D3 correspond to successive layers of cotton after removing the first layer seen in D.

The two adsorbent materials tested were compared in terms of operational aspects and cost. Because cotton is a soft-textured fibrous material, it facilitated the operability of the adsorption column at the time of packaging and unpacking. Almost 100 g (with a cost equivalent to approximately R$ 25.00/kg, which is very cheap - approximately 10% of the cost of silicon dioxide (Celite® 512 medium, R$ 263.00/kg)) was adequate to fill the 0.65 L volume of the adsorption column. In addition, other operational limitations (*i.e.*, the formation of preferred paths, as well as the loss of mass between the internal spaces of the adsorption column covers when collecting the Celite® 512 medium at the end of processing), made this product less preferable compared with cotton.

Another important conclusion regarding the operability of the proposed supercritical fluid extraction process concerned the cleaning step.

The need to reduce these cleaning times and the amount of cleaning solvents required led to the identification of empty spaces (Fig. **8**) where the extracted material accumulated after each processing run. Studies performed by Torres [32] showed a similarity to this case, and therefore, the same strategies, such as the use of Teflon bushings, were applied to improve the equipment. Using Teflon bushings in the separators caused a dramatic drop in both cleaning time, from 40 h (after each experimental run, especially in cases of A. B and C) to 12 h (after each experimental run, D and E), and consumption solvent (essentially ethanol) volumes, from 14 L to 3 L.

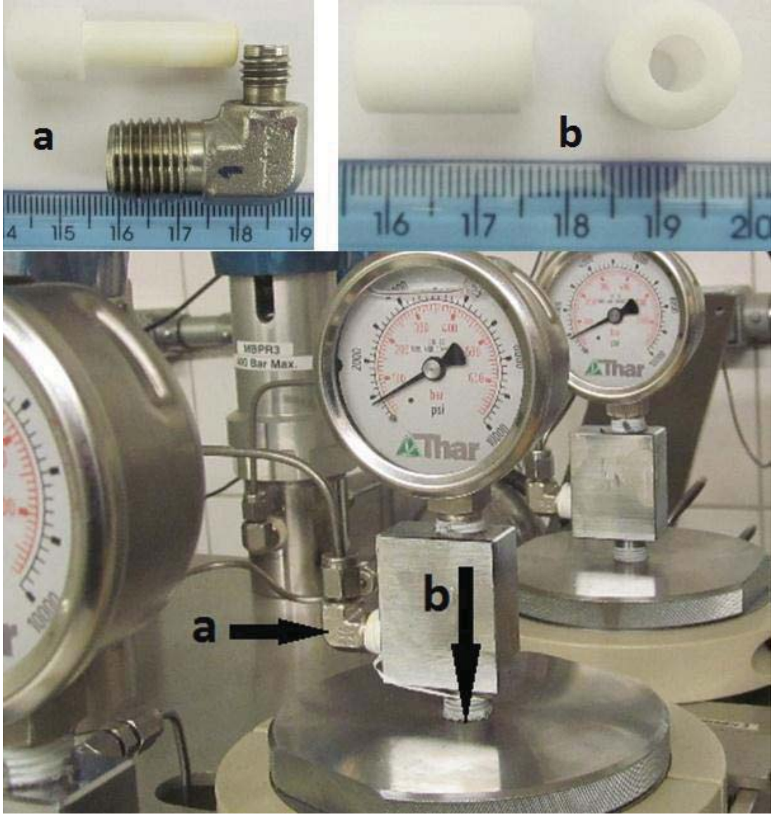

**Fig. (8).** Teflon bushings used to avoid the accumulation of extract in the SFE pilot-scale unit coupled with the developed online subcritical adsorption-based device.

### 3.3. Effects of the Ratio of Solvent Mass to Feed Mass on the Extraction Yield Using the SFE Pilot-scale Unit Coupled with the Online Subcritical Adsorption-based Device

After unit adjustments and the technical viability demonstration of the inclusion of the subcritical adsorption-based device in the SFE pilot-scale unit, 5 experimental runs (A-E) were performed to evaluate the effect of the solvent mass to feed mass (S/F) ratio on the extraction yield to validate the technical viability and better understand the process. Based on the OEC and kinetic parameters calculated using the data from defatting annatto seeds using the SFE bench-scale system, the extraction conditions were selected.

Run A was performed according to the initial state of the pilot-scale unit, *i.e.*, without any changes being made to the equipment. Thus, this extraction process served to allow data such as $CO_2$ consumption (approximately 13 kg) to be surveyed under the processing conditions. In addition, Run A was performed to better compare the effects of the inclusion of the adsorption column and Lung Tank (LT) in assisting $CO_2$ recycling. Runs B and C correspond to the tests in which silicon dioxide (Celite® 512 medium) was used as an adsorbent material. Runs D and E correspond to the tests where the cotton (in a uniform orthopedic blanket roll) was used as an adsorbent material.

Table **3** shows the results for extraction yield and residual extract indicators when S/F was varied. It should be observed here that the extraction yield differs from the global extraction yield (X0) previously obtained in the bench-scale unit since the latter represents the maximum amount of extract that can be recovered from a raw material at a given extraction pressure and temperature (2.82% for annatto seeds for SFE at 313 K and 20 MPa). As observed for the bench-scale unit experiments, the values obtained related to the extract collected during the cleaning of the unit (residual extract amount) were strongly correlated to the S/F ratio. The higher the S/F ratio is, the higher is the extraction yield measured by collecting the extracts in the 3 separation vessels and the lower is the accumulation of extract inside the parts of the SFE pilot-scale unit. Comparing the percentages of residual extract obtained in the pilot-scale unit during runs A-E (7.58-16.65%) with those obtained previously in the bench-scale unit (4.05-8.23%), it can be observed that the pilot-scale unit retains more extract in its tubing (7.58-16.65%). Therefore, any gain related to an increase in the amount of extract collected would be interesting. Therefore, the use of cotton as an adsorbent material for increasing extract recovery in addition to allowing $CO_2$ recycling is a critical issue to be addressed.

In this context, we can see that an increase of 3.8 and 1.6% in the extraction yield was obtained using cotton inside the online subcritical adsorption-based device in runs D and E, respectively. In terms of the adsorbed extract amount (in grams) divided by 100 g of cotton, equivalent values of 1.02% and 0.67% were obtained, indicating that the amount of annatto-seed oil in the final adsorbent material is approximately 1% (see Fig. **7d**, the colored cotton material with extract). Considering that in Run E, the S/F ratio was approximately double the value applied in Run D, we can infer that similarly to what occurred with the residual extract deposited in the tubing lines in both units tested here, the use of a higher S/F ratio is also preferred for reducing extract accumulation inside the adsorption column. In our case, when the S/F ratio was increased by 119.61%, the amount of adsorbed extract was reduced by 34.31%. Table **3** also shows the improvement in terms of the lower retention and accumulation of extracts obtained by the inclusion of Teflon bushings in the unit, comparing runs A, B and C (without unit adjustments) with runs D and E (with Teflon bushings and coupled with the developed online subcritical adsorption-based device.

Del Valle *et al.* [33] mentioned that one of the reasons for a low extraction yield compared with that obtained on an analytical (or laboratory) scale is the dispersion of the material extracted between the extraction column and the separators. Corroborating these findings, in this study, we found that 92.54% and 94.37% of the total residual extract was provided by the separation-vessel cleaning step in runs B and C, respectively.

About the possibility of channeling in this study, it should be noted that the apparent density of the raw materials inside the extractor was the same for the whole runs, 0.7 g/cm3. According to the reference 33, the possibility of channeling is higher for a bed with height per diameter ratio of unity, which is far away from height per diameter ratio of 2.4 to 5.8 in the current study. Homogeneous color of the raw material at the end of the extraction is another reason that $CO_2$ distributed homogeneously inside the bed and channeling problem did not occur.

Table **3** shows that increasing S/F improves the extraction yield (g extract/ 100 g annatto seeds) due to higher $CO_2$ consumption. This table also reports the adsorbed mass of extract that is 1 g, for Run B with S/F of 25.45, and 0.66 g, for Run C with S/F of 55.98. One probable reason for lower amount of adsorbed mass of extract of Run C compared to run B is the higher amount of S/F in Run C that carries out higher amount of the extract available in the adsorption column. Accordingly, the total extraction yield, which includes the mass of extract inside the separators and the mass of extract in the adsorption column, determined to be 1.42, for Run A, 1.89, for Run B, and 2.46, for Run C. Moreover, as shown in the last column of this table, an increment of S/F from 3.69 to 55.98 reduces the amount of extract in the lines from 16.65% to 7.58%. This is mainly due to the employing adsorption-base device together with Teflon bushings in the separators that leads to recovering higher amount of extract and reducing the amount of extract in the lines.

### 3.4. Estimated Cost of the Constructed Online Subcritical Adsorption-based Device

Considering the need to perform tests in the pilot-scale unit to verify the possibility of recirculating $CO_2$ with the least amount of drag on the extract and without requiring a great initial investment, some components were borrowed from other units: a column that was used as adsorption column, a manometer and a micrometering valve (Fig. **9**). Then, corrective maintenance of 4 valves that were previously in disuse was performed; these valves appear with a (*) in

Table **4**. In this sense, disregarding the cost calculation of items with (*), we can say that an initial investment of only USD 461.36 was necessary.

**Fig. (9).** Components of the homemade online subcritical adsorption-based device developed to allow effective $CO_2$ recycling.

**Table 4. Estimated cost of the components of the homemade device developed to allow effective $CO_2$ recycling.**

| Product Name | Qty | Price | Total (USD) |
|---|---|---|---|
| Adsorption column (AdC1)* | 1 | 4,000.00 | 4,000.00 |
| Recording manometer NS163 (Class A2 Ø150 mm, ABNT NBR-14105/98, São Paulo, Brazil)* | 1 | 140.00 | 140.00 |
| Blocking valve (Autoclave Engineers, 10V2071, PA, USA)* | 4 | 166.00 | 664.00 |
| Micrometering valve (Autoclave Engineers, 10VRM2812, PA, USA)* | 1 | 466.53 | 466.53 |
| Heat exchanger with electric resistance to heat the micrometric valve | 1 | 70.00 | 70.00 |
| Tubing 1/8” (6 m) (Fopil, ASTMA269S, Campinas, Brazil) | 1 | 89.62 | 89.62 |
| Ferrule 1/8” (Fopil, Campinas, Brazil) | 5 | 3.24 | 16.2 |
| Connector Tee OD 1/8” (Fopil, ASTMA276, Campinas, Brazil) | 2 | 44.30 | 88.60 |
| Connector Tee side NPT 1/4” (Fopil, A.276316, Campinas, Brazil) | 2 | 20.13 | 40.26 |
| Connector OD-NPT (Fopil, ASTMA269TP316S, Campinas, Brazil) | 2 | 11.80 | 23.60 |
| Silicone hose 14 x 8 x 3 mm (15 m) Ref. MS-207-KNR (Sinergia Científica, Campinas, Brazil) | 1 | 57.74 | 57.74 |
| Diaphragm gas meter Itrón G1.6 DN20 x 110 mm (Hidrovolt, Campinas, Brazil) | 1 | 75.34 | 75.34 |
| | | Total | 5.731,53 |

On the other hand, an estimated total cost of USD 5731.36 would be required to build the developed device in an SFE unit like the one used here.

## CONCLUSION & FUTURE DEVELOPMENTS

The information obtained from the data used to construct the overall extraction curve (OEC) in the SFE bench-scale unit was of great use since it enabled a decision regarding the choice of the extraction time used in the pilot-scale unit for technical validation of the online subcritical adsorption-based device for assisting $CO_2$ recycling. The validation criterion adopted, in addition to visual observation of the adsorbent material after the SFE process, consisted of monitoring the effects of the increase in the solvent mass to feed mass (S/F) ratio on the extraction yield and percentage of residual extract in the equipment parts and comparing the results for the SFE pilot-scale unit coupled with the online subcritical adsorption-based device with the results obtained for the SFE bench-scale unit. In general, the higher the S/F ratio was, the higher was the extraction yield measured and the lower was the accumulation of extract inside the parts of the SFE unit used. Comparing the percentage of residual extract obtained in the pilot-scale unit during runs A-E (6.55-16.65%) with those obtained previously in the bench-scale unit (4.05-8.23%), it can be observed that the pilot-scale unit retained more extract in its tubing (6.55-16.65%).

The amount of extract in each of the parts of the SFE pilot-scale unit reported during the mass balance allowed the recognition of the loss of extract material caused by the existence of empty spaces between connections. This form of loss caused the accumulation of residual extract during the extraction process, and in addition to resulting in low extraction yields, led to a higher consumption of solvents, energy and working hours during the cleaning of the equipment. In this sense, the detailed observation of the connections and various equipment parts suggested the use of an inert material (such as Teflon bushing) to fill these spaces.

The incorporation of an adsorption column along with valves and accessories allowed preliminary tests to be carried out to validate the reincorporation of the $CO_2$ recirculation system in the pilot-scale unit. The tests consisted of defatting annatto seeds, reaching S/F values of 25 and 55 with a mass flow rate of 200 g/min in all cases. This approach achieved the reasonable use of economic resources while at the same time achieving a major saving regarding the purchase of solvent. We could infer that similarl to what occurred with the residual extract deposited in the tubing line in both units tested here, the use of a higher S/F ratio is also preferred for reducing extract accumulation inside the adsorption column.

The cost survey showed that it would cost an estimated total of USD 5731.36 to install the developed device in an SFE unit like the one coupled here (5 L).

Since the proposed device was successfully technically validated using cotton as an adsorbent material, future developments will include the performance of the pseudocontinuous SFE of target compounds using supercritical $CO_2$ in the pilot-scale unit used in this study. Pseudocontinuous SFE is recommended because using n ($n \geq 2$) extractor vessels, an industrial plant enables a pseudocontinuous operation to be simulated by intercalating the charge/extraction/discharge steps of each vessel.

Furthermore, the use of other adsorbent materials that in addition to allowing $CO_2$ recycling, can be further used directly as an adsorbent material enriched in bioactive compounds will be studied. For the case of cotton, the colored material (cotton with extract) is applicable in an extremely limited range of applications, and thus this material is unlikely to be pursued in the following steps.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declares no conflict of interest, financial or otherwise.

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