17TH EUROPEAN MEETING ON SUPERCRITICAL FLUIDS

7TH EUROPEAN MEETING HIGH PRESSURE TECHNOLOGY

Institute of Chemical and Environmental Technology (ITQUIMA)

APRIL 8 - 11, 2019

PROGRAMME

Universidad de Castilla-La Mancha
CAMPUS DE EXCELENCIA INTERNACIONAL

itquima UCLM

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7^{th} European Meeting on High Pressure Technology

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The International Society for Advancement of Supercritical Fluids (ISASF) and the Organizing Committee of the 17th European Meeting on Supercritical Fluids (EMSF) welcomes you to Ciudad Real. This is the 17th of a fruitful serie of meetings, where Scientists, Students and Industry Partners discuss current developments and innovations based on the extraordinary properties of supercritical fluids. The meeting will be held in Ciudad Real (Spain), between 8-11th of April 2019. As in previous years, we will have parallel sessions, plenary lectures, oral communications and poster sessions.

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POSTER PRESENTATIONS

Poster session 1. 8th-9th April.

Poster session 2. 10^{th}-11^{th} April.


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POSTER PRESENTATIONS

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POSTER SESSION 1

8-9 April 2019

SUPERCRITICAL FLUIDS AND AROMATICS PLANTS, A FRAMEWORK TO SHARE RESEARCH AND SOCIAL INCLUSION

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1. Introduction

The SPAGYRIA Project (EU Project € 1.8M) arises from one of the line extension objectives set out in the TECHNOBIOCROP Project of MINECO (0.5 M €) for the use of fractions extracts of natural products obtained through sustainable technologies. Spagyria is also a project of cooperation, solidarity and innovation that aims to create a line of plant extracts for the production of ECO-cosmetics. The project involves seven partners including tutelary associations of groups at risk of social exclusion to help improve their employability. The aim is to capitalize on the experience and economic development of the partners on both sides of the Spanish-French border, both at the level of cultivation and conditioning of aromatic plants and at the level of sustainable extraction and evaluation of prepared products.

Figure 1. Aromatic and medicinal plants cultivated in Huesca (Spain).

2. Results and discussion

At the moment, agronomic trials are being carried out in parallel in three different locations (Huesca, Pamplona and Toulouse) to determine the best growing conditions of eight aromatic and medicinal plants (Figure 1). The first studies of supercritical extraction and concentration of active principles using carbon dioxide as an anti-solvent agent indicate some species of sage, calendula and lemon balm as one of the most suitable for the preparation of ECO-cosmetic ingredients.[1]

3. Conclusions

Although Spagyria is still in an initial development phase we would like to emphasize that it brings together three essential characteristics: collaboration, sustainability and social impact, it is possible that social inclusion and research go together to achieve an egalitarian and knowledge society.

The project has been 65% cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020). POCTEFA aims to reinforce the economic and social integration of the French–Spanish–Andorran border. Its support is focused on developing economic, social and environmental cross-border activities through joint strategies favouring sustainable territorial development.

El proyecto ha sido cofinanciado al 65% por el Fondo Europeo de Desarrollo Regional (FEDER) a través del Programa Interreg V-A España-Francia-Andorra (POCTEFA 2014-2020). El objetivo del POCTEFA es reforzar la integración económica y social de la zona fronteriza España-Francia-Andorra. Su ayuda se concentra en el desarrollo de actividades económicas, sociales y medioambientales transfronterizas a través de estrategias conjuntas a favor del desarrollo territorial sostenible.

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INTRODUCTION, OBJECTIVES AND 1st RESULTS

The SPAGYRIA Project (EU Project € 1.8M) arises from one of the line extension objectives set out in the TECHNOBIOCROP Project of MINECO (0.5 M €) for the use of fractions extracts of natural products obtained through sustainable technologies. Spagyria is also a project of cooperation, solidarity and innovation that aims to create a line of plant extracts for the production of ECO-cosmetics. The project involves seven partners including tutelary associations of groups at risk of social exclusion to help improve their employability. The aim is to capitalize on the experience and economic development of the partners on both sides of the Spanish-French border, both at the level of cultivation and conditioning of aromatic plants and at the level of sustainable extraction and evaluation of prepared products.

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Although Spagyria is still in an initial development phase we would like to emphasize that it brings together three essential characteristics: collaboration, sustainability and social impact, it is possible that social inclusion and research go together to achieve an egalitarian and knowledge society.

Supercritical Extraction Device (left), Extractor and Cyclone Vessels (right). Calendula officinalis extract

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Figure2.-Solid particles micronized from the ethanolic extract of Melissa officinalis. Vessel (rigth).

Figure 1.- Trial plantation. November 2018. ATADES-HUESCA (Spain).

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The organizing committee of the 17th European Meeting on Supercritical Fluids herein certifies that

ANA MARÍA MAINAR FERNÁNDEZ

participated in the meeting held between the 8th and 11th April in Ciudad Real, Spain.

The organizing committee,

Juan Francisco Rodríguez

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Supercritical fractionation of extracts from leaves of *Salvia officinalis*

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1. Introduction

Salvia officinalis (Figure 1), commonly known as *sage*, is a perennial plant that belongs to *Lamiaceae* family, order *Lamiales*, and genus *Salvia*. This plant is native to Mediterranean countries, although is currently cultivated in different regions.[1]

Figure 1. *Salvia officinalis* cultivated in Huesca (Spain).

Salvia officinalis (SO), has been considered as a universal panacea due to its great medicinal properties as antiseptic, anti-inflammatory or stimulant.[1] It is an aromatic plant whose essential oil has also antifungal and antioxidant activities.

Some researchers have demonstrated that the essential oil of the plant has a clear potential to treat the Alzheimer's disease and to improve the memory. All these effects have been attributed to biological active compounds such as monoterpenes, diterpenes, triterpenes and phenolic compounds which can be found in leaves.[1,2]

Then, it is worth to investigate how to obtain, from the extracts of SO leaves, fractions that would be enriched in the bioactive compounds. For this purpose, the Supercritical Antisolvent Fractionation (SAF) technique has been selected because it has been found very effective in the fractionation of this kind of compounds.[3-5] In the SAF process, an ethanolic extract of SO is mixed in a chamber with supercritical CO_2 in such a way that those compounds of the extract that are insoluble precipitate in the chamber whereas other compounds that remain dissolved are recovered in a different vessel downstream. The optimum operation conditions are determined and quality of the fractions is assessed through HPLC analysis.

2. Results and discussion

SO leaves were firstly dried in order to reach a humidity degree near to 11%. Then they were ground and sieved (ANSI/ASAE S319.4 standard) to select an average particle diameter of ≈500 μm. After a defatting step using supercritical CO_2, the exhausted solid vegetable material was soaked in absolute ethanol (1:10) for 48 h to obtain the solution feed for the SAF experiments.

The SAF apparatus basically consists of two main parts: a pumping section and a supercritical fluid separation section. A set of resistances and thermocouples along with a controller allows to regulate the temperature. At the bottom part of the vessel precipitator a metallic filter retains insoluble compounds. A downstream collector retains the organic solvent (ethanol) and the compounds that are still dissolved in the mixture CO_2-solvent. Both of the obtained fractions were analysed by HPLC technique.

A Response Surface Methodology (RSM) using Central Composite Design (CCD) was used to design a set of experiments with the aim of determining the effect on the fraction yield of two factors (pressure and CO_2 flow rate) and so to identify optimum working conditions. The software MINITAB® was used for data analysis and modelling.

Table 1. Experimental Central Composite Design (CCD) for SAF process.

Run Order	1	2	3	4	5	6	7	8	9	10	11	12	13
Pressure (CO_2) (bar)	115	90	115	115	80	150	115	115	90	115	140	140	115
CO_2 Mass flow rate (g min^{-1})	35	17	10	35	35	35	35	35	53	60	53	17	35

Table 1 shows the different working conditions for pressure and CO_2 flow rate according to a CCD experiment design. As it is tabulated, the experiments are carried out sequentially. Experiment working conditions range for pressure, between (80-150 bar) and in the case of mass flow between (10-60 g/min).

HPLC analyses were carried out in an e-Alliance 2695 System HPLC-PDA (Waters Technologies). A CORTECS® column 18, 4.6 x 150 mm and particle size of 2.7 µm. The mobile phase flow, 0.8 mL/min, consisted of three solvents: (A) water, (B) acetonitrile and (C) water–acetic acid (0.1%). Separations were performed at room temperature and the injection volume was 10 µL.

3. Conclusions

Salvia officinalis leaves present a moderate yield for ethanol soaking process once nonpolar compounds have been previously extracted with supercritical CO_2 so that it turns to be an interesting candidate for further SAF process. In order to achieve the maximum yield in the fractionation process the optimum working conditions for pressure and CO_2 flow rate were determined. The HPLC analysis proved that it is possible a good enrichment in the bioactive compounds of interest.

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Introduction and Objectives

Salvia officinalis (Figure 1), commonly known as sage, is a perennial plant that belongs to *Lamiaceae* family, order Lamiales, and *genus Salvia*. This plant is native to Mediterranean countries, although is currently cultivated in different regions.[1]

Salvia officinalis, (SO), has been considered as a universal panacea due to its great medicinal properties as antiseptic, anti-inflammatory or stimulant.[1] It is an aromatic plant whose essential oil has also antifungal and antioxidant activities.

The essential oil of the plant has a clear potential to treat the Alzheimer's disease and to improve the memory. All these effects have been attributed to bioactive compounds such as monoterpenes, diterpenes, triterpenes and phenolic compounds which can be found in leaves.[1,2]

The aim of this work is to obtain, from the extracts of SO leaves, fractions of enriched bioactive compounds. For this purpose, the Supercritical Antisolvent Fractionation (SAF) technique has been selected.[3-5] In the SAF process, an ethanolic extract of SO is mixed in a chamber with supercritical CO_2 and those extract compounds that are insoluble precipitate in the chamber whereas other dissolved compounds are recovered downstream. The optimum operation conditions are determined and the quality of the fractions is assessed through HPLC analysis.

Figure 1. Salvia officinalis cultivated in Atades-Huesca (Spain)

Table 1. Experimental design (CCD): experiment order, values of the variables and yields in the precipitator (Y_p), collector (Y_C) and total yield (Y_{Total}).

Experim. Number	*P* (bar)	*Q* $_{CO_2}$ (g/min)	Y_p (%) w/w	Y_c (%) w/w	Y_T (%) w/w
1	92	53	53.0	12.9	65.9
2	120	35	62.8	15.4	78.3
3	120	60	59.4	16.3	75.7
4	120	10	54.6	27.8	82.4
5	92	17	64.7	17.5	82.3
6	120	35	64.8	17.4	82.2
7	148	53	57.2	17.8	75.0
8	80	35	61.9	7.1	69.0
9	160	35	57.4	20.6	77.9
10	148	17	55.8	28.4	84.2
11	120	35	62.6	22.5	85.1
12	120	35	60.0	20.8	80.8
13	120	35	61.7	19.9	81.6
14	120	35	61.0	17.7	78.7
15	120	35	60.7	22.6	83.2

Material Pretreatment

Drying - Crushing and sieving (ANSI/ASAE S319.4 standard) - Defatting - Soaking:

Figure 2. Moisture analyzer (left), sieving machine (center) and soaking (right)

Fractionation, Experimental Design and Results

Equipment: lab scale SAF apparatus (Fig. 3). Precipitator retains the insoluble compounds. Collector recovers an ethanolic solution of the compounds soluble in the mixture CO_2-ethanol.

Figure 3. Left: Scheme of the SAF plant. CO_2 pump (PCO_2), solution pump (PLIQ), refrigerating bath (CB), heat exchanger (HE), precipitator (E), automatic back-pressure regulator (ABPR), manual back-pressure regulator (MBPR), collector (S), Bourdon (M), Thermocouple (T); : view of the SAF plant. At the right: some solid extract fractions obtained at the precipitator chamber.

- Operation conditions: solution composition: 3% (w/w) of extract (soaking) in ethanol; solution flow rate: 0.45 mL/min and precipitator temperature: 40 ºC
- Experimental design: Response Surface Methodology (RSM) using Central Composite Design (CCD) for pressure, *P* and CO_2 flow rate, Q_{CO_2} (***Table 1***)

Conclusions

Salvia officinalis leaves present a moderate yield for ethanol soaking process once nonpolar compounds have been extracted with supercritical CO_2 so that it results an interesting candidate for SAF process. The optimum working conditions for pressure and CO_2 flow rate were determined for the maximum yield. The HPLC analysis indicated a good enrichment in the bioactive compounds of interest.

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Teresa Vélez, Raquel Mur, Carlota Giménez-Rota, Elisa Langa, José F. Martínez-López, Ana M. Mainar
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Introduction and Objetives
Salvia officinalis (Figure 1), commonly known as sage, is a perennial plant that belongs to Lamiaceae family, order Lamiales, and genus Salvia. This plant is native to Mediterranean countries, although is currently cultivated in different regions.
Salvia officinalis, (SO), has been considered as a universal panacea due to its great medicinal properties as antiseptic, anti-inflammatory or stimulant. It is an aromatic plant whose essential oil has also antifungal and antioxidant activities.
The essential oil of the plant has a clear potential to treat the Alzheimer's disease and to improve the memory. All these effects have been attributed to bioactive compounds such as monoterpenes, diterpenes, triterpenes and phenolic compounds which can be found in leaves.
The aim of this work is to obtain, from the extracts of SO leaves, fractions of enriched bioactive compounds. For this purpose, the Supercritical Antisolvent Fractionation (SAF) technique has been selected. In the SAF process, an ethanolic extract of SO is mixed in a chamber with supercritical CO2 and these extract compounds that are insoluble precipitate in the chamber whereas other dissolved compounds are recovered downstream. The optimum operation conditions are determined and the quality of the fractions is assessed through HPLC analysis.
Figure 1. Salvia officinalis cultivated in Atades-Huesca (Spain)
Material Pretreatment
Drying - Crushing and sieving (ANSI/ASAE S319.4 standard) - Defatting - Soaking
Fractionation, Experimental Design and Results
Equipment: lab scale SAF apparatus (Fig. 3). Precipitator retains the insoluble compounds. Collector recovers an ethanolic solution of the compounds soluble in the mixture CO2-ethanol.
Conclusions
Salvia officinalis leaves present a moderate yield for ethanol soaking process once nonpolar compounds have been extracted with supercritical CO2 so that it results an interesting candidate for SAF process. The optimum working conditions for pressure and CO2 flow rate were determined for the maximum yield. The HPLC analysis indicated a good enrichment in the bioactive compounds of interest.
References
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The organizing committee of the 17th European Meeting on Supercritical Fluids herein certifies that

JOSÉ FRANCISCO MARTÍNEZ LÓPEZ

participated in the meeting held between the 8th and 11th April in Ciudad Real, Spain.

The organizing committee,

Juan Francisco Rodríguez

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Supercritical fractionation of extracts from flowers of *Calendula officinalis*

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1. Introduction

Calendula officinalis is an annual herbaceous plant that belongs to *Asteraceae* family, order *Asterales*, and genus *Calendula*. This plant is native to Europe, more specifically to the South and the West. It prefers clay soils and is a plant of temperate climates, but can withstand frost and droughts. [1]

Calendula officinalis (CO), produces an ample set of phyto-chemical compounds that include carbohydrates, amino acids, lipids and fatty acids, carotenoids, terpenoids, flavonoids, quinines, coumarins, etc. whose amount depends on the time and place of cultivation. Several of these compounds show pharmacological activities such as antibacterial, anti-inflammatory, immuno-stimulant, hepatoprotective, anti-HIV, etc. [2] Then, the study of the extracts of the plant and either the separation of the active compounds or the enrichment of a fraction in those compounds from those extracts is of special relevance in order to study the possibility of developing new drugs.

As previous experiments have demonstrated that the flowers of CO contain the greater part of the compounds of interest and in greater proportions[2] this study is aimed to carry out a fractionation of extracts from flowers of CO in order to obtain fractions enriched in those compounds. For this purpose, the Supercritical Antisolvent Fractionation (SAF) technique using CO_2 as antisolvent has been selected because it has been found very effective in the fractionation of bioactive compounds.[3-5] SAF is based on the complete miscibility between the selected liquid solvent and the supercritical fluid, at the process conditions; whereas, the solutes have to be not soluble in the supercritical medium. Therefore, when the liquid solution (solvent plus solutes) is sprayed in the precipitator, the liquid is rapidly extracted by the supercritical fluid (antisolvent) and the solute precipitates as a powder at the bottom of the vessel. The optimum operation conditions are determined, and quality of the fractions is assessed through HPLC analysis.

2. Material and methods

2.1. Material treatment

CO flowers were firstly dried in order to reach a humidity degree near to 11%. Then they were crushed and sieved (ANSI/ASAE S319.4 standard) to select an average particle diameter of ≈500 μm. The next step was extracting the lipids compounds (degreasing) of the plant using supercritical CO_2. Later, this material was macerated in absolute ethanol (1:10) for 48 h to obtain the solution feed for the SAF experiments.

2.2. Supercritical antisolvent fractionation process (SAF).

The SAF apparatus consists of two main parts: a pumping section and a supercritical fluid separation section. The temperature control is achieved by means of resistances, thermocouples and a controller. At the bottom part of the precipitator a metallic filter retains the insoluble compounds. A downstream collector is used to recover the organic solvent (ethanol) and the compounds that are still dissolved in the mixture CO_2-solvent. Both of the fractions obtained were analyzed by HPLC.

A Response Surface Methodology (RSM) using Central Composite Design (CCD) was employed to determine the effect of two factors (pressure and CO_2 flow rate) on the fraction yield and to identify the optimum working conditions. For data analysis and model establishing the software MINITAB® was used.

2.3. HPLC Analyses

The HPLC analyses were carried out in an e-Alliance 2695 System HPLC-PDA (Waters Technologies). The used column was a CORTECS® 18, 4.6 x 150 mm and particle size of 2.7 µm. The mobile phase flow, 0.8 mL/min, consisted of three solvents: (A) water, (B) acetonitrile and (C) water–acetic acid (0.1%). Separations were performed at room temperature and the injection volume was 10 µL.

3. Results and Discussion

The degree of humidity obtained from the samples is close to the 11% which is the limit established to continue with the process. To carry out the experiment, 500 g of the flowers were ground until obtaining a particle size of approximately 500 µm and 5 extractions of 100 g each were performed with supercritical CO_2. Waxy compounds and essential oil were obtained as a result of each one of the extractions, leaving the powder of the flowers defatted (exhausted calendula) ready to macerate with ethanol.

Then, the maceration of each exhausted calendula obtained in each experiment was carried out in a bottle with absolute ethanol (1:10) for 48 h at room temperature and under stirring (Image 1).

Image 1. Maceration of exhausted calendula with ethanol (1:10)

After the maceration, all the content was filtered, obtaining a final approximate volume of 5400 mL with a concentration of 0.006 g/mL, that is, with 32.54 g of extract from CO flowers. With this data, the yield after degreasing and maceration with absolute ethanol under these conditions turned out to be 7.2%.

4. Conclusions

Calendula officinalis flowers present a moderate yield the maceration with ethanol, once nonpolar compounds have been previously extracted with supercritical CO_2, so that this material appears as an interesting candidate for further SAF process.

The optimum conditions of pressure and CO_2 flow rate in the fractionation in order to achieve a maximum yield were determined. From the obtained results, SAF process could be considered as an efficient fractionation technique (in the found optimal working conditions) for ethanol extracts from *Calendula officinalis* flowers. The HPLC analysis proved that it is possible a good enrichment in the bioactive compounds of interest.

The project has been 65% cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020). POCTEFA aims to reinforce the economic and social integration of the French–Spanish–Andorran border. Its support is focused on developing economic, social and environmental cross-border activities through joint strategies favouring sustainable territorial development.

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SUPERCRITICAL FRACTIONATION OF EXTRACTS FROM FLOWERS OF *Calendula officinalis*

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Introduction

Calendula officinalis **(CO) is an annual herbaceous plant of *Asteraceae* family whose flowers (Fig. 1) contain an ample set of phyto-chemical compounds, several of which have been reported to show pharmacological activities such as antibacterial, anti-inflammatory, anti-HIV, immuno-stimulant, hepato-protective, etc.[1]**

Figure 1. Flowers of *Calendula officinalis.*

Objective

As Supercritical Antisolvent Fractionation (SAF) has been found very effective in the fractionation of bioactive compounds,[2,3] the aim of this work is to separate extracts of flowers of CO in fractions enriched in those compounds by applying SAF using CO_2 as antisolvent.

Fractionation and experimental design

- ✓ **Material: flowers of CO, dried (humidity 11 %), crushed and sieved (average particle size ≈ 500 μm) ad degreased with supercritical CO_2.**
- ✓ **Extract: maceration of material in ethanol (1:10 w/w) for 48 h.**
- ✓ **Equipment (Fig. 2): lab scale SAF apparatus. Precipitator retains the insoluble compounds. Collector recovers an ethanolic solution of the compounds soluble in the mixture CO_2-ethanol.**

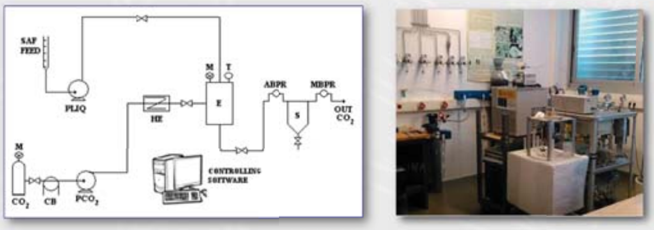


Figure 2. Left: Scheme of the SAF plant. CO_2 pump (PCO_2), solution pump (PLIQ), refrigerating bath (CB), heat exchanger (HE), precipitator (E), automatic back-pressure regulator (ABPR), manual back-pressure regulator (MBPR), collector (S), Bourdon (M), Termocouple (T); Right: view of the SAF plant.

- ✓ **Experimental design (Table 1): Response Surface Methodology (RSM) using Central Composite Design (CCD) for precipitator pressure (P) and CO_2 flow rate (Q_{CO2}).**
- ✓ **Data analysis and model establishing: software MINITAB®.**

Table 1. Experimental design (CCD): experiment order, values of the variables and yields (percentage of mass with respect to the mass of extract feeded) in the precipitator (Y_P), collector (Y_C) and total yield (Y_{Total}).

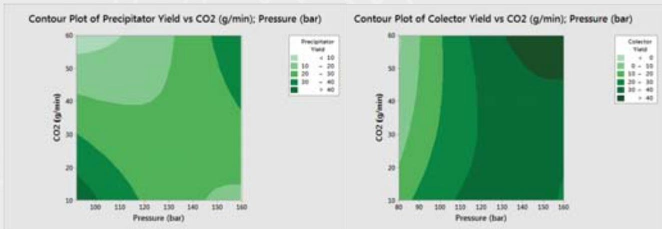
Exp. Number	P / bar	Q_{CO2} /g/min	Y_P / % w/w	Y_C / % w/w	Y_{Total} / % w/w
1	160	35	25.4	34.7	60.1
2	120	35	28.8	15.8	44.6
3	120	35	29.1	26.8	55.9
4	92	17	41.9	22.0	63.9
5	148	53	31.7	43.8	75.5
6	120	35	18.6	34.3	52.9
7	120	35	15.6	31.4	47.0
8	148	17	24.5	32.7	57.2
9	80	35	56.3	1.4	57.7
10	92	53	11.3	15.3	26.7
11	120	35	13.9	30.2	44.1
12	120	60	12.6	31.0	43.6
13	120	10	26.6	32.9	59.6
Solution composition: 3% (w/w) of extract (maceration) in ethanol Solution flow rate: 0.45 mL/min Precipitator temperature: 40 °C					

Results and discussion

- ✓ **Yield for maceration is 7.2 % with respect to the mass of CO flowers.**
- ✓ **Yields in the precipitator, collector and the total yield (sum of yields in precipitator and collector) are shown in Table 1.**
- ✓ **The precipitate was mainly an oily liquid with little powder (Fig. 3).**

Figure 3. Left: liquid from the precipitator (diluted with ethanol); Center: liquid from the collector; Right: view of the injector in the precipitator with a little powder.

- ✓ **Every yield has been fitted to a full quadratic equation (MINITAB®). Contour plots of the fitting appear in Fig. 4.**



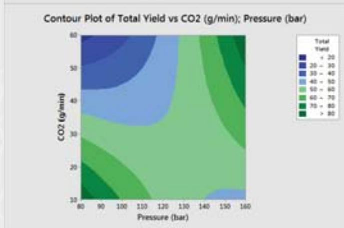


Figure 4. Contour plots for each yield according to the fitting to a full quadratic equation

- ✓ **The optimal conditions for Y_P are P = 92 bar and Q_{CO2}= 10 g/min whereas for Y_C and Y_{Total} as well as for all of the yields simultaneously are P = 160 bar and Q_{CO2}= 60 g/min. Higher values would be worth to be tested as they could improve the yields.**
- ✓ **Preliminary chemical HPLC analysis of fractions points to a good separation of bioactive compounds of interest.**

References

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Introduction
Calendula officinalis (CO) is an annual herbaceous plant of Asteraceae family whose flowers (Fig. 1) contain an ample set of phyto-chemical compounds, several of which have been reported to show pharmacological activities such as antibacterial, anti-inflammatory, anti-HIV, immuno-stimulant, hepato-protective, etc.
Figure 1. Flowers of Calendula officinalis.
Objective
As Supercritical Antisolvent Fractionation (SAF) has been found very effective in the fractionation of bioactive compounds, the aim of this work is to separate extracts of flowers of CO in fractions enriched in those compounds by applying SAF using CO2 as antisolvent.
Fractionation and experimental design
Material: flowers of CO, dried (humidity 11 %), crushed and sieved (average particle size ≅ 500 μm) ad degreased with supercritical CO2.
Extract: maceration of material in ethanol (1:10 w/w) for 48 h.
Equipment (Fig. 2): lab scale SAF apparatus. Precipitator retains the insoluble compounds. Collector recovers an ethanolic solution of the compounds soluble in the mixture CO2-ethanol.
Results and discussion
Yield for maceration is 7.2 % with respect to the mass of CO flowers.
Yields in the precipitator, collector and the total yield (sum of yields in precipitator and collector) are shown in Table 1.
The precipitate was mainly an oily liquid with little powder (Fig. 3).
Figure 3. Left: liquid from the precipitator (diluted with ethanol); Center: liquid from the collector; Right: view of the injector in the precipitator with a little powder.
Every yield has been fitted to a full quadratic equation (MINITAB®). Contour plots of the fitting appear in Fig. 4.
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The optimal conditions for YP are P = 92 bar and QCO2 = 10 g/min whereas for YC and YTotal as well as for all of the yields simultaneously are P = 160 bar and QCO2 = 60 g/min. Higher values would be worth to be tested as they could improve the yields.
Preliminary chemical HPLC analysis of fractions points to a good separation of bioactive compounds of interest.
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Supercritical Antisolvent Fractionation of *Jasonia glutinosa* Extracts

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1. Introduction

In recent years chemistry industry has focused on developing alternative processes with a less significant environmental impact. As is well known, supercritical fluids are receiving great interest from various industrial sectors due to their versatility and being a prominent part of the so-called green solvents. In fact, due to their properties, they allow to perform separation, extraction, fractionation, synthesis or precipitation processes effectively, which generates value-added products for industries such as pharmaceutical, cosmetic or agri-food.[1]

Figure 1. *Jasonia glutinosa*

The aim of this study is to evaluate the supercritical anti-solvent fractionation (SAF) with CO_2 to concentrate compounds of interest from extracts of *Jasonia glutinosa* (Figure 1).[2] *Jasonia* belongs to the family *Asteraceae*, subfamily *Asteroideae*. More commonly known as rock tea or Aragon tea, it is a perennial plant up to 30-40 cm with yellow tubular flowers and glandular hairs that produce a resinous substance with a characteristic odor. It grows in high mountain areas (1300-2000 m) mainly in cracks of dry and sunny limestone crags. This plant species can be found in the western Mediterranean area, mainly in the eastern half of the Iberian Peninsula.[3]

The composition of the essential oil of *Jasonia glutinosa* is headed by camphor (30-40%) and borneol (15-20%) and followed by other minor compounds such as cyclic monoterpenes, oxygenated monoterpenes, sesquiterpenes, flavonoids and phenolic acids.[4,5] These minority compounds have properties of pharmacological interest as anti-inflammatory, antimicrobial or antioxidant. [6]

2. Results and discussion

Pressure and flow rate of CO_2 have been selected as factors under study, keeping constant the temperature (40 ˚C) and the flow rate of the feed solution (0.45 mL / min). The minimum and maximum levels of the factors considered were (80-150) bar and (10-60) g / min. To find the optimal conditions of the process, an experimental design was initially carried out using statistical software. Response surface methodology (RSM) using central composite design (CCD) was used to evaluate the effect of pressure and CO_2 flow rate on the SAF process. Table 1 shows the number of experiments performed and the working conditions set for each of them.

A previously defatted ethanolic tincture of *Jasonia* was used as a feed solution in order to generate concentrated extracts in polyphenolic derivatives such us chlorogenic acid. This compound is the ester of caffeic acid and quinic acid, which acts as an intermediate in the biosynthesis of lignin. It is long known as an antioxidant and also slows the release of glucose into the bloodstream after a meal.

Table 1. Experimental Central Composite Design

N. Exp.	*P* (bar)	QCO_2 (g/min)
1	140	17
2	90	53
3	80	35
4	115	35
5	115	35
6	115	60
7	90	17
8	115	35
9	150	35
10	115	10
11	115	35
12	115	35
13	140	53

The HPLC analyses were carried out in an e-Alliance 2695 System HPLC-PDA (Waters Technologies). The used column was a CORTECS® 18 2.7 µm (4.6 x 150 mm). The mobile phase flow, 0.8 mL/min, consisted of three solvents: (A) water; (B) acetonitrile and (C) water–acetic acid (0.1%). Separations were performed at 30 ± 5 ˚C and the injection volume was 10 µL. A gradient elution was performed to change the strength of the eluent during the analyses.

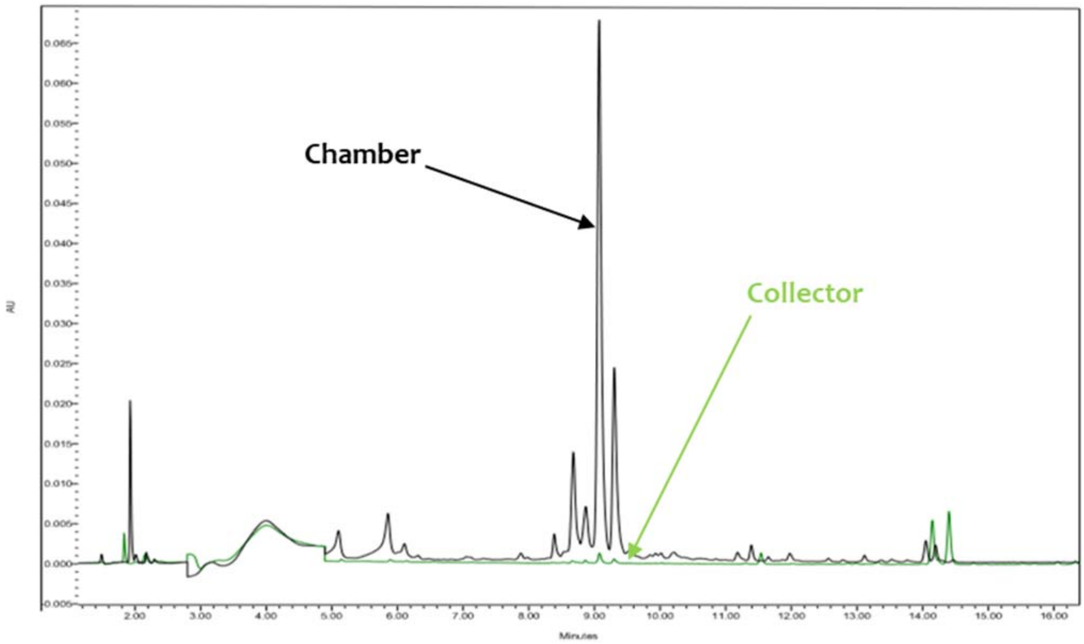

Figure 2. Chromatogram of the two fractions obtained in the SAF process: camera (black) and collector (green)

In the SAF process, the total feeding material recuperation yield is reached up to 95%. The yield of precipitation in chamber vary from 32% to 64%. HPLC-PDA results showed that the two fractions obtained in the SAF process provide different composition, Figure 2, with enrichment in chlorogenic acid in the precipitation chamber. The statistical analysis of the results obtained shows the dependence of the yields of the process with the two variables studied, pressure and flow of CO_2

3. Conclusions

Experimental design has been used to optimize an anti-solvent precipitation technique. The results achieved evidences that it is possible to fractionate satisfactorily the extracts of *Jasonia glutinosa*. Performing an HPLC analysis of the 3 samples (feed, chamber and collector) has shown that the chlorogenic acid is retained exclusively in the chamber, thus obtaining a fraction enriched in a known antioxidant.

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Supercritical antisolvent fractionation of *Jasonia glutinosa* extracts

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Introduction

Jasonia belongs to the family *Asteraceae*, subfamily *Asteroideae*. More commonly known as rock tea or Aragon tea, this plant species can be found in the western Mediterranean area, mainly in the eastern half of the Iberian Peninsula.[1] The composition of the essential oil of *Jasonia glutinosa* is headed by camphor (30-40%) and borneol (15-20%) and followed by other minor compounds such as cyclic monoterpenes, oxygenated monoterpenes, sesquiterpenes, flavonoids and phenolic acids.[2,3] These minority compounds have properties of pharmacological interest as anti-inflammatory, antimicrobial or antioxidant.[4]

The aim: To evaluate the supercritical anti-solvent fractionation (SAF) with CO_2 to concentrate compounds of interest from extracts of *Jasonia glutinosa*

Methods and materials

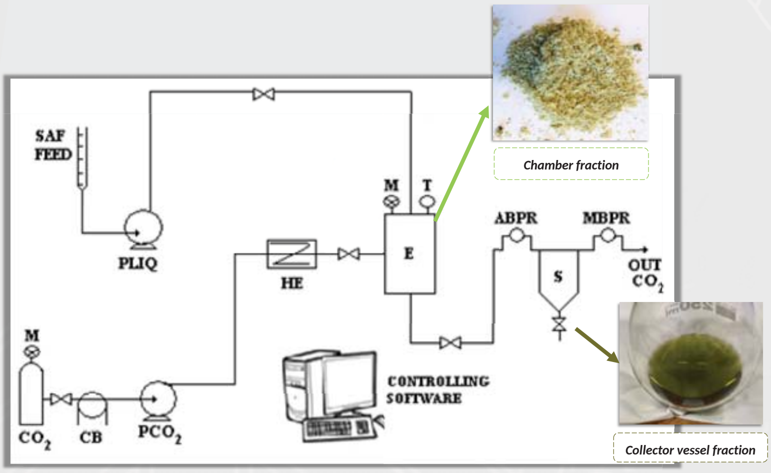

Fig. 1 – SAF equipment diagram. Scheme of the SAF plant. CO_2 pump (PCO_2), solution pump (PLIQ), refrigerating bath (CB), heat exchanger (HE), precipitator (E), automatic back-pressure regulator (ABPR), manual back-pressure regulator (MBPR), collector (S), Bourdon (M), Thermocouple (T).

J. glutinosa feed extract solution was concentrated in two fractions using the SAF technique, with the equipment of Figure 1. Both fractions, the chamber and collector vessel fraction, were analysed with HPLC (Fig 3). Table 1 shows the experiments realized and their experimental conditions. Figure 2 shows the material obtained in the experiment one.

Table 1.- Experimetal conditions and order accordign to the experimental desing.

Exp. Number	P (bar)	Q CO_2 (g/min)
1	140	17
2	90	53
3	80	35
4	115	35
5	115	35
6	115	60
7	90	17
8	115	35
9	150	35
10	115	10
11	115	35
12	115	35
13	140	53

Feed extract solution: 3% (w/w) of extract (maceration) in ethanol
Solution flow rate: 0,45 mL/min
Precipitation chamber temperature: 40 °C

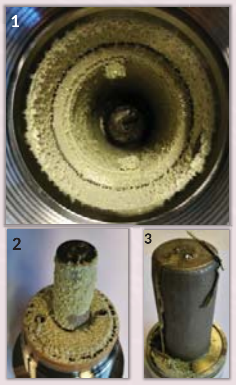

Fig. 2 – Powder from chamber (1) and filter (2 and 3)

Results

- In the supercritical anti-solvent fractionation process, the total feeding material recuperation yield is reached up to 95%. Table 2 shows the values of yields of each experiment.
- Two compounds from phenolic acids family are identified: chlorogenic acid and gallic acid.
- Performing an HPLC analysis of the 3 samples (feed, chamber and collector) has shown that the chlorogenic acid is retained exclusively in the chamber, thus obtaining a fraction enriched in a known antioxidant.
- The statistical analysis shows that the yields of the process depends on caudal and pressure of CO_2. Optimized values to this process are 15 g/min of CO_2 caudal and 150 bar of CO_2 pressure.

Table 2. - Values of the yields (percentage of mass with respect to the mass of extract feeded) in the precipitation chamber (Y_P), collector (Y_C) and total yield (Y_{Total}); and values of concentration of chlorogenic acid in chamber (% chamber / % feed solution).

Exp. Number	Y_P (%) w/w	Y_C (%) w/w	Y_{Total} (%) w/w	Concentration factor
1	64,4	30,9	95,3	4,1
2	48,0	23,3	71,3	4,1
3	32,4	20,4	52,8	3,1
4	52,4	15,6	68,0	5,0
5	47,7	26,4	74,1	4,5
6	54,7	29,9	84,6	3,7
7	41,3	24,8	66,1	4,4
8	46,6	24,6	71,3	4,8
9	52,7	22,8	75,5	5,8
10	46,7	30,7	77,4	4,5
11	42,2	27,1	69,3	4,5
12	46,2	26,8	73,0	3,8
13	48,7	26,2	74,9	4,0

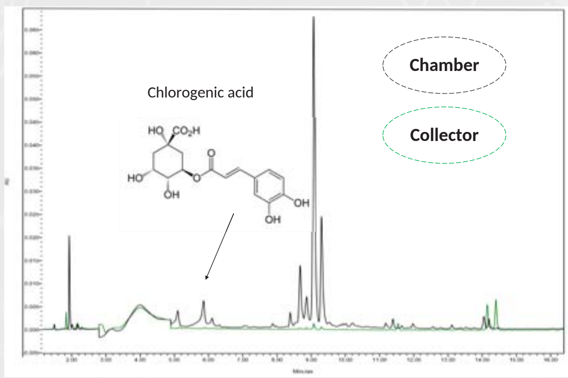

Fig. 3 – Chamber (black) and collector vessel (green) chromatogram.

Acknowledgments:

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17th European Meeting on SUPERCRITICAL FLUIDS

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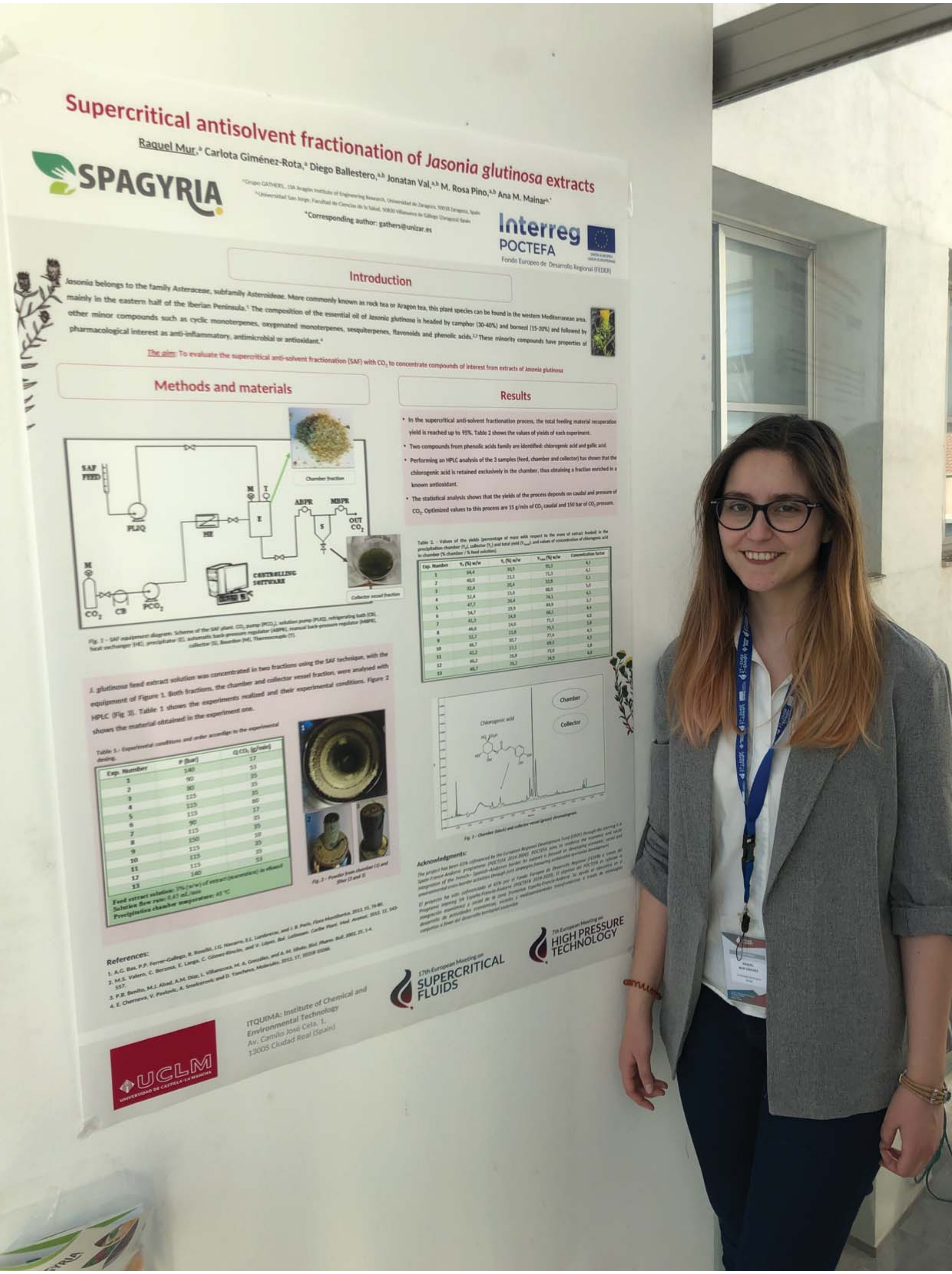
Supercritical antisolvent fractionation of Jasonia glutinosa extracts
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The organizing committee,

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