2º Encontro Ibérico de **Fluidos Supercríticos**
Encuentro Ibérico de **Fluidos Supercríticos**

EIFS2022
Coimbra- Portugal

Book of abstracts

28 Fevereiro - 2 Março
28 Febrero - 2 Marzo

Book of abstracts

This book contains the abstracts presented at the Second Iberian Meeting on Supercritical Fluids (2º Encuentro Ibérico de Fluidos Supercríticos/2º Encontro Ibérico de Fluidos Supercríticos), held in Coimbra – Portugal, on 28 February-2 March 2022.

Second Iberian Meeting on Supercritical Fluids
(2º Encuentro Ibérico de Fluidos Supercríticos/
2º Encontro Ibérico de Fluidos Supercríticos)

Book of Abstracts

Second Iberian Meeting on Supercritical Fluids
28 February – 2 March 2022
Faculty of Sciences and Technology
Universidade de Coimbra

Created by:
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Welcome Message

Following the success of EIFS2020, in Santiago de Compostela, it is with great honor and pleasure that we host EIFS2022, the 2nd Iberian Meeting on Supercritical Fluids (2º Encontro Ibérico de Fluidos Supercríticos/2º Encuentro Ibérico de Fluidos Supercríticos), and welcome all participants to Coimbra, Portugal.

EIFS2022 is organized by the Green and Sustainable Processes Lab (GSP Lab @PPB-CIEPQPF, Universidade de Coimbra, Portugal), by the I+D Farma research group (Universidade de Santiago de Compostela, Spain), by PRODEQ (Associação para o Desenvolvimento da Engenharia Química, Coimbra, Portugal), and by Flucomp (Asociación de Expertos en Fluidos Comprimidos, Spain).

Like in the first edition of EIFS, the main goal of EIFS2022 is the dissemination of the high quality R&D&I currently being carried out in Spain and Portugal on a wide range of fundamental and applied topics related to supercritical and high pressure fluids. This event also aims to bring together, to strengthen and to create new ties and collaborations between the Iberian research community and companies working around these topics, as well as to support young Iberian researchers working on these fields. Nevertheless, EIFS2022 official language is English since this event has also contributions from researchers all over the world.

EIFS2020 took place on the cusp of the emergence of the COVID-19 pandemic. As a consequence of it, these last two years have been extremely challenging for all of us. We were confined at home and our laboratories were closed for long periods of time. We were able to gather and discuss research work with our students and colleagues, but in ways that were certainly not the most efficient and desirable.

This had a great impact on all our research activities. But we adapted and overcame all these difficulties. In this way, the EIFS2022 Organizing Committee also considers that this event could also symbolically represent a turning-point, a “victory” over this virus, and the return to the personal and professional “normality” that we have all longed for a long time.

In addition, EIFS2022 will be held in parallel to the “Spanish-Portuguese Industry-Academia Aerogel Meeting", organized by AERoGELS CA18125 COST Action (https://cost-aerogels.eu/), and to the "Workshop on Green Processes and Technology", organized by Greenering CA18224 COST Action (https://www.greenering.eu/). Given the important role and relevance of supercritical fluid technologies in aerogel processing and green processes, we foresee clear synergies between both events and interesting outputs from discussions between their participants.

Another novelty of EIFS2022 is a short training course (5 h) especially intended for the PhD students attending the event. This course will cover fundamental

topics, selected applications and economics on supercritical fluid technology and aspires to train the next-generation of experts in the field.

We are pleased to provide a remarkable scientific programme and hope that EIFS2022 will offer the opportunity to discuss scientific and technological issues.

The Organizing Committee gratefully acknowledges all authors for their contributions and all sponsors for their generous financial support.

We wish you an excellent conference both scientifically and socially, and an enjoyable stay in Coimbra.

28 February 2022
EIFS2022 Organizing Committee

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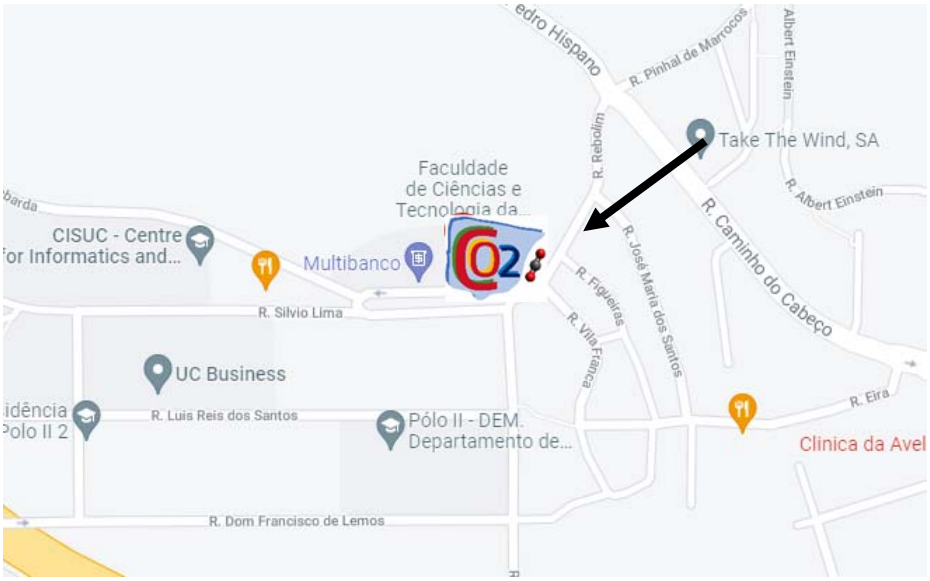
Carlos Silva (Univ. Aveiro)

Practical information

Conference location

Faculdade de Ciências e Tecnologia (Main Building) (Pólo II)
Universidade de Coimbra (UC)
R. Sílvio Lima, Pólo II, 3030-790 Coimbra, Portugal
Google Maps: https://goo.gl/maps/MkEyzyvsuLGCL1WZ9



Tourist information

Coimbra is one of the most historic cities in Portugal. Located at the margins of the Mondego river. It is rich in centuries-old monuments and worldwide famous for its university, which is one of Europe's oldest universities. The University of Coimbra (UC) was created in 1290 and includes ten organic units of teaching and research (eight faculties: Arts and Humanities, Law, Medicine, Sciences and Technology, Pharmacy, Economics, Psychology and Educational Sciences, Sport Sciences and Education Physics, as well as the Institute for Interdisciplinary Research, the College of Arts, and two research units: European Judicial University Court and Institute of Nuclear Sciences Applied to Health. UC also comprises a set of academic extension structure units such as the Botanical Garden, the General Library, the University's Press, the Science Museum, the April 25 Documentation Centre, the Gil Vicente's Academic Theatre, the University Stadium, the Geophysical Institute, the Natural History Museum and the Astronomical Observatory, etc. All these structures are distributed by three campi (Pólo I, Pólo II, and Pólo III). In addition, UC is one of the UNESCO's World Heritage sites since 2013 and enrols several UNESCO chairs. EIFS2022 will be held in Coimbra at the Faculty of Sciences and Technology of the University of Coimbra (FCTUC). FCTUC is one of UC's faculties and institutes, and its research activities are framed into more than 20 Research Units and Associated Research Units, which are widely recognized references in a vast number of scientific and technological fields.

How to arrive from the airport

HOW TO ARRIVE FROM PORTO AIRPORT:
The airport serving the city of Porto is the International Airport Francisco Sá Carneiro (https://www.aeroportoporto.pt), which is located about 11 km north of the city centre. There are direct and non-direct flights to Porto from anywhere in the world.
One of the easiest ways to get to Coimbra from Porto Airport is using the Porto Metro (https://www.metrodoporto.pt), and then to catch a train to Coimbra: Porto Airport → Metro to Campanhã Train Station → Train to Coimbra
For this, you should take the Metro Purple Line (Line E) to Trindade Metro Station, then switch to Orange Line (Line F), or Blue Line (Line A), or Red Line (Line B), or Green Line (Line C), to get to Campanhã Train Station. At Campanhã Train Station, you should catch a Train to Coimbra (Coimbra-B Station) (https://www.cp.pt)
In addition, Porto airport has various public/private transport links and car rental services.

HOW TO ARRIVE FROM LISBON AIRPORT:
The airport serving the city of Lisbon is the International Airport Humberto Delgado (https://www.aeroportolisboa.pt). There are direct and non-direct flights to Lisbon from anywhere in the world. The airport has various public/private transport links and car rental services.
One of the easiest ways to get to Coimbra from Lisbon Airport is using the Lisbon Metro (https://www.metrolisboa.pt), and then to catch a train to Coimbra: Lisbon Airport → Metro to Oriente Train Station → Train to Coimbra

For this, you should take the Metro Red Line to Oriente Metro Station. Then, at Oriente Train Station, you should catch a Train to Coimbra (Coimbra-B Station) (https://www.cp.pt)

HOW TO ARRIVE BY CAR
It is quite easy to get to Coimbra by car. There are plenty of online guides to find out how to drive to Coimbra. The fastest way is to use Highway A1 and then exit at Exit Coimbra Norte or Exit Coimbra Sul.

Full Conference Programme

Monday, 28.Feb.2022

13:00 - 14:00	**Registration**		
14:00 - 14:30	**Welcome/Opening Session**		
	Plenary lecture		
14:30 - 15:30	PL-01	Nanoparticle processing with compressed CO_2 - basic and applied research	Nora Ventosa
Session 1	**Co-chairs: Elena Ibáñez (CIAL-CSIC); Mara Braga (Univ. Coimbra)**		
15:30 - 15:50	O-01	Scale-up of supercritical CO_2 extraction process for production of milk thistle extract	Stoja Milovanovic
15:50 - 16:10	O-02	Use of supercritical CO_2 to obtain enriched flavouring fractions from black truffle	Eva Tejedor-Calvo
16:10 - 16:30	O-03	Supercritical CO_2 selective extraction of saponins with nutraceutical application towards metabolic syndrome from germinated black beans (*Phaseolus vulgaris L.*)	Daniel Guajardo-Flores
16:30 - 17:00	**Coffee Break + Poster Session**		
	Keynote Lecture		
17:00 - 17:30	K-01	Application of supercritical carbon dioxide in catalysis and in aerogel fabrication	Pedro Vidinha
Session 2	**Co-chairs: Ana M. Mainar (Univ. Zaragoza); Naiara Fernández (iBET)**		
17:30 - 17:50	O-04	Supercritical CO_2 extraction as a tool to isolate phytochemicals from Rice (*Oryza sativa L.*) by-products	João P. Baixinho
17:50 - 18:10	O-05	Extraction from hemp biomass and seed	Lourdes Calvo
18:10 - 18:30	O-06	Supercritical fluid extraction and fractionation of the oil from marina microalgae *Crypthecodinium cohnii* to obtain biodiesel and omega-3 compounds (DHA)	Beatriz P. Nobre
18:30 - 20:30	**Welcome Reception**		

Tuesday, 1.March.2022

	Plenary lecture		
09:00 - 10:00	PL-02	"Greenering" pharmaceutical manufacturing: the role of $scCO_2$	Ana Aguiar-Ricardo
Session 3	**Co-chairs: María José Cocero (Univ. Valladolid); José Coelho (I.S. Engenharia Lisboa)**		
10:00 - 10:20	O-07	Accessing cork biomass fractions via conventional chemical and high pressure and temperature water	Elaine G. Mission
10:20 - 10:40	O-08	Subcritical water extraction of quercetin derivatives and other flavonoids from onion skin wastes ("Allium cepa" cv. Horcal): effect of temperature and solvent properties	Óscar Benito-Román
10:40 - 11:00	O-09	Novel micelles of PEG functionalized with coumarin toward multifunctional drug delivery systems	Sonia López
11:00 - 11:30	**Coffee Break + Poster Session**		
	Keynote Lecture		
13:30 - 12:00	K-02	12 years of the Sudden Expansion Reactor: Invention, Key Results, Opportunities and Industrial Experience	Danilo Cantero
Session 4	**Co-chairs: Ignacio Gracia (U. Castilla-La-Mancha); Ana Aguiar-Ricardo (U. Nova Lisboa)**		
12:00 - 12:20	O-10	Soybean oil-based cyclic carbonates synthesis in supercritical CO_2 sustained by halide organocatalysts	Juan Catalá
12:20 - 12:40	O-11	High pressure phase equilibria prediction of renewable lignin derived aromatic compounds with CO_2	Selva Pereda
12:40 - 13:00	O-12	Machine learning models for the prediction of diffusivities in supercritical CO_2 systems	José P.S. Aniceto
13:00 - 14:30	**Lunch**		

Plenary lecture			
14:30 - 15:30	PL-03	Sustainable aerogel production using SICLA technology	Eunate Goiti & Francisco Ruiz
Session 5		**Co-chairs: Albertina Cabañas (U. Complutense Madrid); Ana V.M. Nunes (U. Nova Lisboa)**	
15:30 - 15:50	O-13	Batch and continuous production of directly compressible drug nanopowders using CO_2-assisted spray coating	Luís Padrela
15:50 - 16:10	O-14	Micronization of Ciprofloxacin by the Supercritical Antisolvent Technique (SAS)	Albertina Cabañas
16:10 - 16:30	O-15	Manufacturing of liposomal-loaded hydrophilic or hydrophobic drugs dry powder formulations using supercritical carbon dioxide	Clarinda Costa
16:30 - 17:00	**Coffee Break + Poster Session**		
Session 6		**Co-chairs: Lourdes Calvo (U. Complutense Madrid); Ana L. Oliveira (U. Católica Portuguesa)**	
17:00 - 17:20	O-16	Supercritical CO_2 technology for the production of sterile and drug-loaded scaffolds	Víctor Santos-Rosales
17:20 - 17:40	O-17	Development of bioMIPs Using Supercritical Fluids	Ana I. Furtado
17:40 - 18:00	O-18	Customized and bioactive bone scaffolds obtained by a novel technological duo	Ana Iglesias-Mejuto
18:00 – 22:00	**Cultural Activies & Meeting Dinner**		

Wednesday, 2.March.2022

Plenary lecture			
09:00 - 10:00	PL-04	Challenge dots for Supercritical community: Flucomp rocks!	Ignacio Gracia
Session 7		**Co-chairs: Sagrario Beltrán (Univ. Burgos); Carlos Silva (Univ. Aveiro)**	
10:00 - 10:10	SO-01	Combined supercritical fluid technology for the preparation of dry powders loaded with beclomethasone dipropionate for pulmonary delivery	Thoa Duong
10:10 - 10:20	SO-02	Supercritical fluid extraction of lupane triterpenoids from "Acacia dealbata" biomass – effect of pressure, temperature and cosolvents	Vítor H. Rodrigues
10:20 - 10:30	SO-03	Polymorphic control of indomethacin with the gas antisolvent method	Fidel Mendez Canellas
10:30 - 10:40	SO-04	Towards the fabrication of medicinal nanovesicular formulations under GMPs	David Piña Muñoz
10:40 - 10:50	SO-05	Self-assembled silk fibroin aerogel particles for wound healing	Beatriz G. Bernardes
10:50 - 11:00	SO-06	Supercritical carbon dioxide Salvia miltiorrhiza extraction: process optimization and analysis of bioactive compounds	Encarnación Cruz
11:00 - 11:30	**Coffee Break + Poster Session**		
11:30 -12:30	FLUCOMP Meeting		
12:30 – 13:00	**Closing Session & Farewell**		

Plenary Lectures

Molecular nanoparticle processing with compressed CO_2: basic and applied research

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ABSTRACT

Molecular nanoparticles, such as nanovesicles, solid lipid nanoparticles and polymeric micelles, are promising multimolecular assemblies for the development of several type of products like therapeutics, imaging agents, vaccines, cosmetics, agrochemicals and nutraceuticals, due to their great versatility respect to size, composition, surface characteristics and capacity for integrating active ingredients, such as therapeutic or sensing molecules. Besides, they are well recognized as drug delivery systems because of their biocompatibility, biodegradability and low toxicity [1-3]. Many of these molecular nanoparticles are in clinical use as vaccines and drugs [4].

Despite their versatility, a high control and characterization of particle to particle heterogeneity is necessary for an optimal performance of nanoparticle based formulations. Thus, the formation stage of these supramolecular entities must be tightly controlled in order to achieve a homogeneous assembling of the different molecular components constituting the nanoparticle nanostructure. For instance, one of the difficulties for the translation to the clinics of nanoparticle based pharmaceutical formulations, are the difficulties in the scale-up and GMP manufacturing [4].

In my lecture I will focus on the contribution that processes, based on the use of compressed CO_2, are doing to bring molecular nanoparticle based nanomedicines from the lab to the clinics [5-7]. I will present some nanoparticle-based nanomedicines for therapy and diagnosis, produced in our lab, which are currently developed in the frame of interdisciplinary research projects [8-12].

ACKNOWLEDGEMENTS

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"Greenering" pharmaceutical manufacturing: the role of $scCO_2$

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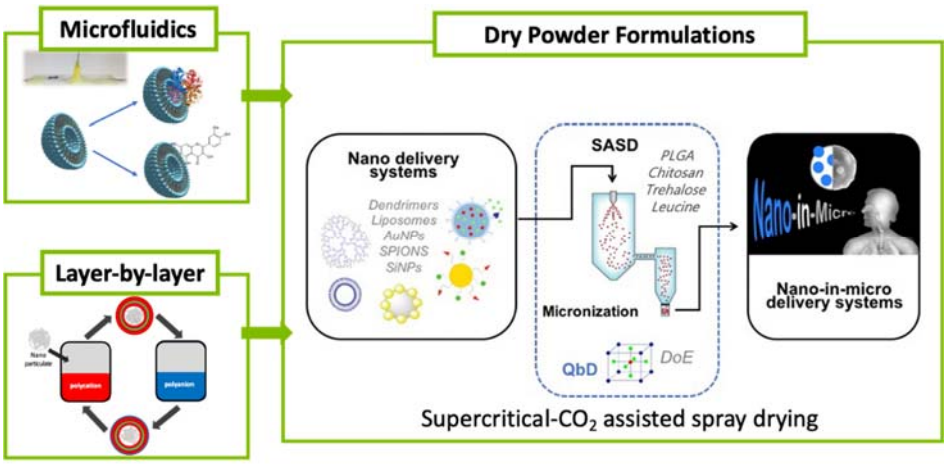
GRAPHICAL ABSTRACT



ABSTRACT

The need for sustainable approaches is invading all fields and steps of manufacturing. From the choice of a green solvent and natural materials to the use of safe chemical reactions and processes, many efforts are described in literature. Supercritical CO_2 technologies have demonstrated utility for the development of improved formulations for pharmaceutical, food and cosmetic applications [1].

This lecture will address specific challenges on the bottom-up assembling of complex composite formulations. Special attention will be given to the integration of supercritical fluid assisted spray drying with other technologies for the generation of precision formulations, for the delivery of small molecule therapeutics to biologic drugs and nucleic acids, namely:

i. Nano-in-microparticles solid dosage forms for pulmonary delivery [2,3]
ii. Micronized layer-by-layer siRNA powders [4]
iii. Nano-in-micro POxylated polyurea dendrimer solid dosage forms [5]
iv. Liposomal dry powder formulations with hydrophobic and hydrophilic compounds [6]

Our results demonstrate the potential of $scCO_2$-assisted technologies for particle engineering of pharmaceutical materials, especially in the manufacture of pulmonary drug delivery systems, considering the tight requirements necessary for the final aerosol properties.

ACKNOWLEDGEMENTS

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020 & UIDP/50006/2020 and PTDC/BII-BIO/30884/2017. AAR acknowledges also CA18224 GREENERING ("Green Chemical Engineering Network towards upscaling sustainable processes"). COST Actions are funded within the EU Horizon 2020 Programme.

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Sustainable aerogel production using SICLA technology

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GRAPHICAL ABSTRACT

Schematic of closed-loop circular economy business model of high-added value products for energy efficiency.

ABSTRACT

Climate change and environmental degradation represent a threat that Europe and the rest of the world are facing. In this regard, Europe has defined a new sustainable strategy called the European Green Deal [1] with the aim of transforming the European Union (EU) into a modern, resource-efficient and competitive economy, where there are no net greenhouse gas (GHG) emissions by 2050 and where economic growth is decoupled from resource use. Therefore, EU has established a roadmap with actions that aim to: i) boost resource efficiency by shifting to a clean and circular economy and ii) restore biodiversity and reduce pollution.

The principal goal is to make the European Union (EU) climate neutral by 2050. This requires actions in all sectors of the economy, including: ***(i)*** EU climate ambition for 2030 and 2050. In this sencse,the new Circular Economy Action Plan has been launched [2]. ***(ii)*** Energy and resource efficient building and renovation: ensuring that buildings are more energy efficient.

Buildings account for 40% of total energy consumption and about 75% of them are energy inefficient. The European Commission is increasingly committed to the energy rehabilitation of the Member States' building stock with the aim of reducing the EU's total energy consumption by 5-6% and reducing CO_2 emissions by 5% [3]. One of the simplest ways to achieve this “energy performance” target is to reduce their energy consumption by decreasing heating and cooling energy demands. Thus, building effective insulation represents an immense market worldwide, for both new constructions and for renovation.

In this perception, silica aerogel, the most effective materials known for thermal insulation at ambient conditions, stands out as the most promising high-performance insulation material. However, the market for aerogels as building-insulation materials remains largely underdeveloped due to the high cost of precursor (~80%) associated with industrial scale production. Thus, price of silica aerogel is clearly the biggest entry barrier of this material in the sector.

KEEY Aerogel and TECNALIA present a **novel sustainable aerogel production technology** called **SICLA™** that follows a closed-loop circular economy model: high-performance building insulation material is manufactured from silica containing waste materials. Silica aerogel materials with exceptional thermal conductivities (λ<0.015 W/mK) are achieved through this novel process. Moreover, the **SICLA™** technology guarantees at least a 40% cost reduction of the aerogel production process.

The present communication attempts to describe different aspects of the process and its implementation, comprising:

- The technology behind the recycling process and manufacture of superinsulating aerogel materials.

The application of **SICLA™** on the design and development of intermediate and semi-finish insulation products for the retrofitting and new insulation market.

ACKNOWLEDGEMENTS
This work was partly supported by the project "Cost-effective recycling of CDW in high added value energy efficient prefabricated concrete components for massive retrofitting of our built environment" -VEEP- (Project number: 723582) and is financed by the European Union under the Horizon 2020 Framework Programme.

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"Challenge dots for Supercritical community: Flucomp rocks!"

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GRAPHICAL ABSTRACT

ABSTRACT

Despite Supercritical Technology is a relatively mature science, their commercial applications are still scarce. Since Kemer NATO meeting, Flucomp has grown up to lead international research in topics like extraction, fractionation, particle formation, sterilization, biopolymers, catalysis, controlled release, Biorefinery or Fodomics, among others. This presentation analyzes the current situation of our community under several frameworks such as *research trends, market demands, legislation opportunities,* or *analytical and technical tools*. Some successful examples are presented, and potential challenge dots are pointed out as possible focus for development of our community with real impact in society.

ACKNOWLEDGEMENTS

Flucomp (especially supporters), AERoGELS and GREENERING COST Actions

Keynote Presentations

Application of supercritical carbon dioxide in catalysis and in aerogel fabrication

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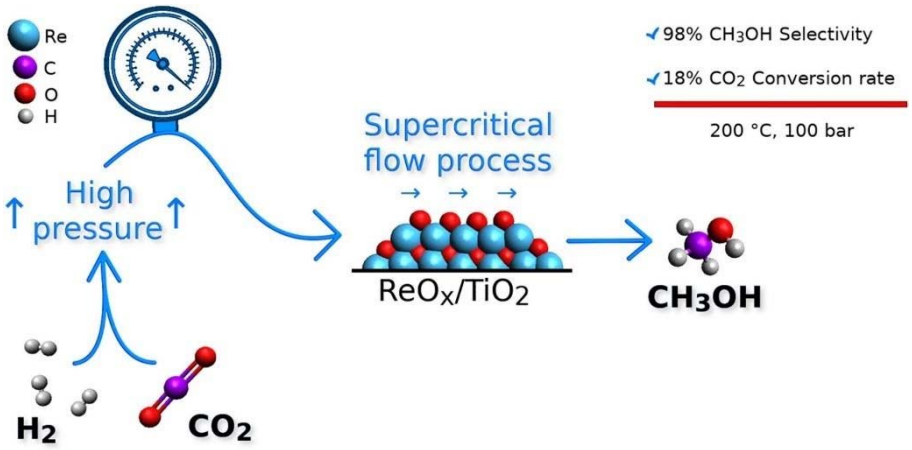
GRAPHICAL ABSTRACT



ABSTRACT

The aim of this presentation is to give an overview of what we are developing in our research group (https://www.suscarbon.com/) regarding the use of supercritical carbon dioxide. In this particularly we are focused in two main subjects: the fabrication an impregnation of starch aerogels [1] and the development of different catalytic processes under supercritical conditions [2]–[4]. Regarding aerogels we develop a one-pot approach to fabricate and impregnate starch aerogels. In this case, we have performed several modifications on the fabrication process in order to integrate gelatinisation, retrogradation and drying with the impregnation process. Therefore, a different drying approach based on supercritical extraction was proposed, as well as the fabrication of starch aerogels in the presence of CO_2. Regarding the development of catalytic processes our main goal is the valorisation of CO_2. In this case we have been using both homogeneous and heterogenous catalysts to hydrogenate CO_2 to alcohols. In this case we a used a series of water-soluble organometallic iridium complexes to achieve the conversion of CO_2 into alcohols near room temperature. In this work, we have shown that $[(\eta^5\text{-}C_5Me_5)Ir(phen\text{-}X)Cl]^+Cl$ (X = NO_2, H or NH_2) were able to hydrogenate CO_2 to methanol, ethanol and iso-propanol in water and supercritical CO_2 without using any additive or activator and at

40 °C. Moreover, a feasible mechanism for the CO_2 conversion to methanol was proposed based on density functional theory (DFT) calculations. Moreover, we also proposed an efficient supercritical flow process for the selective valorisation of CO_2 into methanol under supercritical conditions [3]. At optimized conditions, rhenium oxide on titanium dioxide ($ReOx/TiO_2$) catalyst converts CO_2 into methanol with 98% selectivity and at 18% CO_2 conversion rate at 200 °C, 100 bar and CO_2/H_2 ratio of 1/4. A higher conversion of 41% could be achieved at 250 °C, but the selectivity towards methanol decreases to 64%. This strategy has enabled the development of an efficient high-pressure flow process without compromising methanol selectivity. Moreover, a scale-up project is being proposed for this process as well as a creation of startup – "CARBONIC".

ACKNOWLEDGEMENTS

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12 years of the Sudden Expansion Reactor: Invention, Key Results, Opportunities and Industrial Experience

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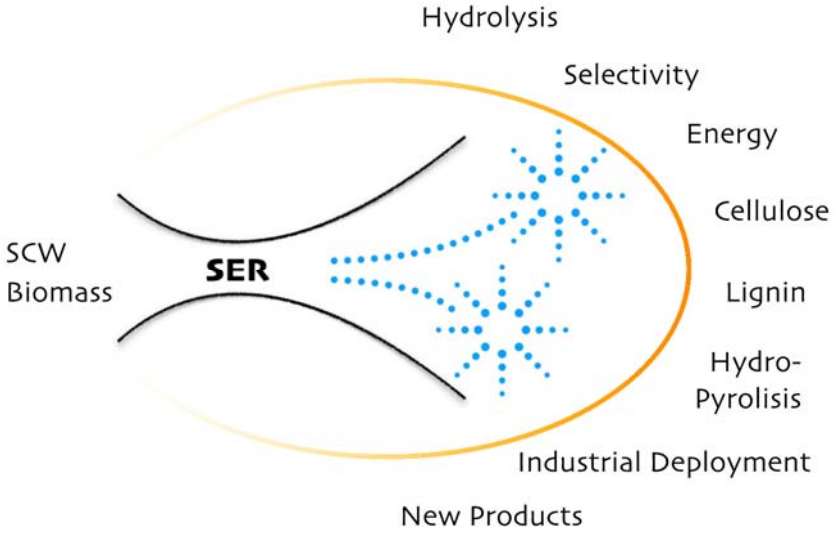
GRAPHICAL ABSTRACT



ABSTRACT

It was back in 2009 when the scientists at the University of Valladolid, Spain, opened a new research program about Supercritical Water Hydrolysis (SCW) of Biomass. The team got inspired by previous publications of Dr Sasaki in the field [1]. The fact that cellulose seemed to swell and partially solubilize in SCW made it a very promising technology to produce cellulosic sugars. It was our goal to employ that concept while maximizing the sugars yields and product concentration. This was the *genesis* of the Sudden Expansion Reactor (SER) [2]. The main goal for the development was to achieve the highest rates of heating, reaction, and cooling. In that way, the reactions can be accelerated to intensify the process while keeping high yields. This device was achieved by linking a biomass-SCW mixer, a tubular reactor and a let-down valve. Special attention should be paid to the mixer (short reaction times require very efficient mixing) and the let-down valve (the valve should be able to reduce the pressure and handle solids at the same time). With this device, we were able to execute reactions with reaction times as low as 0.1 seconds at 400 Celsius and 250 bars. More noticeably, this device was able to drop the temperature of the reaction from 400 Celsius to 100 Celsius almost instantaneously (Joule-Thompson effect). This invention allowed many advances in the field, which will be briefly described next. Also, few opportunities to further progress the technology will be presented.

The SER was deeply studied for cellulose and biomass hydrolysis [3–5]. One finding was the optimization of cellulose hydrolysis reaction over glucose and oligosaccharides degradation. That optimization at very low reaction times was translated into cellulose hydrolysis yields higher than 80%. That behavior was also seen for cellulose in lignocellulosic biomass, at lower yields though, around 70%. In the later years, the SER was employed to partially hydrolyze cellulose and produce micro size particles [6]. Those particles are still in development for its best application. SER was also employed for glucose conversion into aldehydes and lignin depolymerization [3,4]. In this case, the reaction times are usually larger, in the order of seconds. Very interestingly, the products profile of the hydrolysis to those components is very similar to the ones obtained from pyrolysis. It becomes then very interesting to evaluate if the water as reaction medium is offering any medium benefit or if it is only an energy carrier. At industrial scale, the company Renmatix is the pioneer in the use of SCW to hydrolyze biomass. The company counts with more than 100 patents, which protects the technology [7]. The author spent 6 years as scientist applying the process intensification concepts of SCW hydrolysis.

Although the technology has been developed deeply, few aspects should still be addressed to fully deploy the technology at efficient and integrated products: (1) Refining: the SH product consists of a mix of mono and oligosaccharides and byproducts. This should be converted to glucose in an efficient and clean way; (2) Since the SER allows extremely low reaction time, could it be used for more labile carbohydrates, like starch or gums? (3) The water and energy demand for this technology is high. It is then very important to develop water recycling systems as well as energy integration loops. Few opportunities were already evaluated for energy integration employing gas turbines. What about Supercritical Water Oxidation?

ACKNOWLEDGEMENTS

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Oral Sessions

Scale-up of supercritical CO_2 extraction process for production of milk thistle extract

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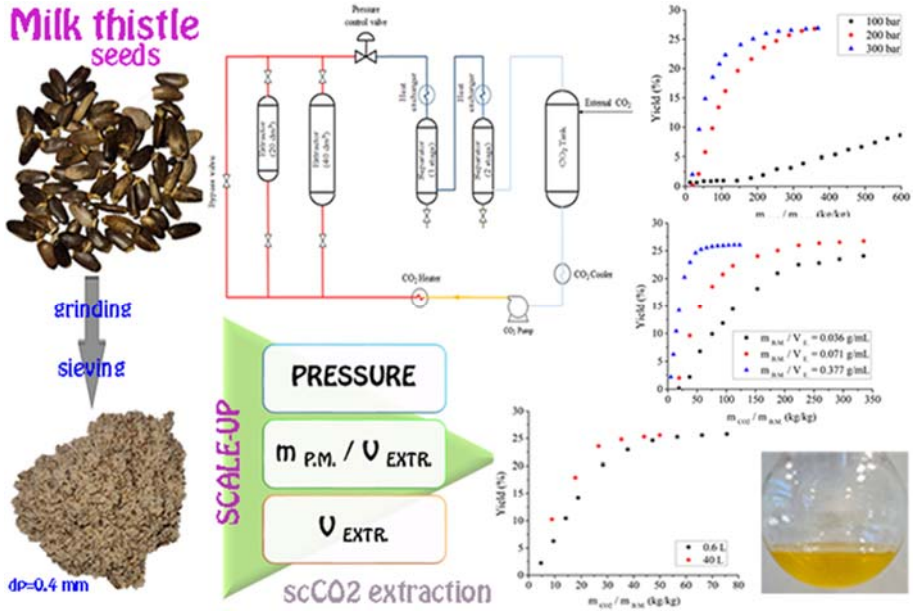
GRAPHICAL ABSTRACT



ABSTRACT

Milk thistle (*Silybum marianum*, Asteraceae) is a plant used in traditional medicine from ancient times mostly for treatment of liver disorders [1,2]. Nowadays it is an industrially valuable crop for preparation of herbal supplements reaching total sales of 16 million USD in the USA market [2]. The separation of valuable bioactive compounds from plant materials can be performed by several methods using organic and inorganic solvents. Nevertheless, supercritical fluid extraction (SFE) that employs CO_2 is considered a green alternative to other conventional extraction methods. In order to be economically viable, the SFE process should be optimized by selection of appropriate pressure and temperature conditions followed by a scale-up. The scale-up of the SFE process can be performed by keeping constant: the ratio of $scCO_2$ flow rate to mass of bed raw material, the ratio of bed length and bed diameter or dimensionless number such as Reynolds number [3]. This study presents the scale-up of the SFE process, first by selection of optimal pressure (100, 200 and 300 bar) at moderate temperature of 40 °C using a lab scale unit (0.6 L),

following by selection of optimal CO_2 consumption per used plant material as well as optimal ratio of plant material mass per volume of extractor. Optimal parameters were further tested using a semi-industrial scale unit (40 L). The highest extraction yield obtained was 27%. The performed scale-up study showed a good agreement between lab and pilot scale units. The qualitative analysis performed using GC/MS showed that obtained extracts contained around 80% of unsaturated fatty acids. The quantitative analysis performed using GC/FID confirmed that predominant fatty acids were oleic and linoleic acids with a content in the range of 191-234 mg/g and 445-514 mg/g, respectively (depending on the SFE pressure). Total phenolic compounds were estimated in each extract by the Folin–Ciocalteu assay reaching ca. 90 mg GAE/g. The free radical scavenging capacity was analyzed by the DPPH assay showing value of IC_{50} (concentration of extract required for the 50% decrease in absorbance of the blank) to be ca. 13 mg/mL.

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Use of supercritical CO_2 to obtain enriched flavouring fractions from black truffle

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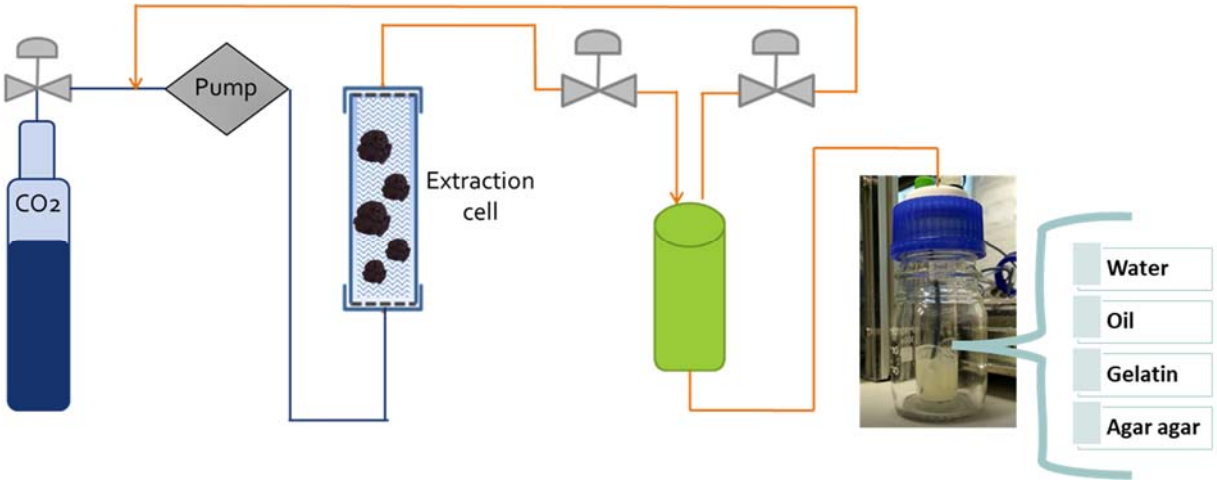
GRAPHICAL ABSTRACT



ABSTRACT

Truffles are a well-known worldwide product mainly appreciated for their unique aroma, which is composed of more than 50 volatile compounds. In the recent years, popularity of truffle products has highly increased in restaurants and supermarkets. Usually the artificial aroma, 2,4-dithiapentane or bis(methylthio)methane, is used as added aroma. however this molecule is not present in the black truffle [1]. To this day, no one has accomplished to find the aromatic extract that evokes the real smell of truffles to use it as food flavoring.

Recently, a new methodology to obtain aromatic compounds from truffles using supercritical CO_2 has been described by our research group [2]. In our studies, parameters like time, pressure and flow rate were optimized, and the addition of grapeseed oil into the separators as a trapping material was studied. This extraction method showed that oil matrix improved trapping some key truffle aromatic compounds such as 2,3-butanodione, 2-methyl-1-butanol, octanal and dimethyl disulphide. Furthermore, olfactometry study helped to detected key aromatic compounds in the truffle extracts.

Apart from aromatic compounds, it is known that some lipidic compounds, such as fatty acids and sterols, are related with truffle aroma and can contribute with flavour attributes.

In that sense, increase their extraction yield might enhance the flavouring properties, as well as the aromatic compounds. For that, after developing the extraction methodology, more improvements were made. Some trapping materials (oil, gelatin, water and agar-agar) were added to the methodology in order to enhance the flavouring compounds retention. In that case anelectrospray ionization quadruple time-of-flight mass spectrometry (UHPSFC/ESI-QTOF-MS) was used to detected more than 30 lipidic compounds. Among them ergosterol, brassicasterol, ergosta-7,22-dienol, oleic and linoleic acid were found in the extracts in high quantities. Also, the use of trapping material allowed capturing higher range of sterols in truffle extracts. This new method has been applied for different truffles species (*Tuber aestium, Tuber indicum and Terfezia claveryi*) with positive results.

According to the current truffle categorization (UNECE Standard FFV-53) [3], only physical aspects were considered to evaluate truffle quality. Therefore, the non-classified truffles, mainly because damage or small size, are categorized as low quality and achieve minor prizes. So, it is a potential source of chemical and aromatic compounds that can be revalorized. For the first time, these truffles were used to obtain a natural enriched fraction with flavouring properties.

ACKNOWLEDGEMENTS

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Supercritical CO_2 selective extraction of saponins with nutraceutical application towards metabolic syndrome from germinated black beans (*Phaseolus vulgaris L.*) by-products

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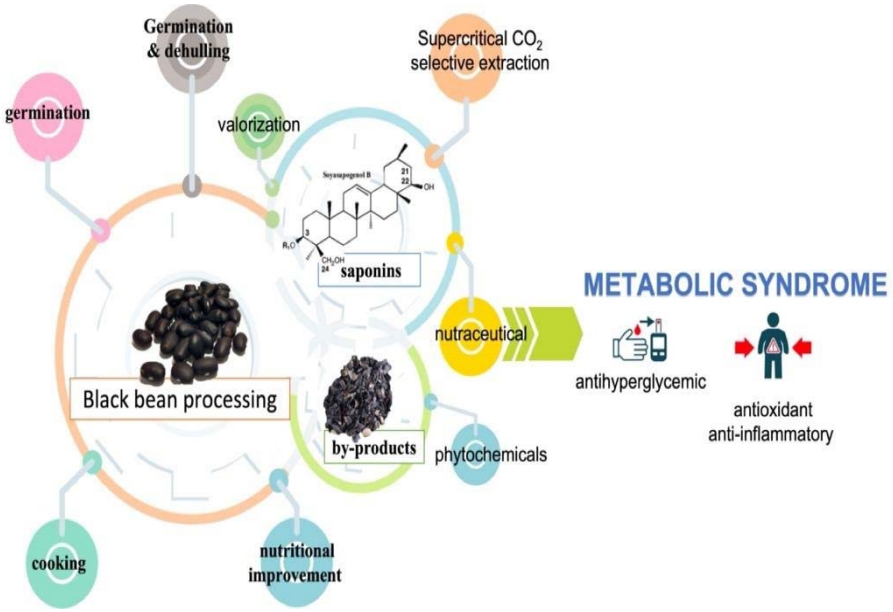
GRAPHICAL ABSTRACT



ABSTRACT

Black beans (*Phaseolus vulgaris L.*) are highly consumed throughout the world mainly for their high protein content. In addition, biologically active substances are found in lower proportions. These main bioactive compounds include saponins, phytosterols, flavonoids and anthocyanins [1,2]. These high value-added compounds have well-known health benefits, such as antioxidant, antiproliferative, anti-inflammatory activity in prostate, liver, mammary, and colon cancer cells, hypocholesterolemia, and inhibitory effects in the absorption of lipids [2–5]. However, the nutritional use of black beans requires a transformation prior to their consumption to enhance their macronutrients digestibility also affecting the content of these minor biologically active substances [6–8]. Their processing causes the generation of by-products with high potential as functional ingredients with nutraceutical properties for pharmaceutical, cosmetic, and biotechnological applications [1,7,9]. Thus, the aim of this research was to evaluate the

use of supercritical carbon dioxide (SC-CO_2) extraction as a selective and environmentally friendly alternative to obtain high-value compounds such as saponins from a black bean (*Phaseolus vulgaris L.*) by-product. A Box-Behnken design was used to evaluate the effect of temperature (40, 55 and 70 °C), pressure (10, 25, 40 MPa), and co-solvent (5, 10 and 15 mL/min) on the soyasaponins recovery. The highest soyasaponin extraction was obtained at 70°C, 10 MPa and 10% co-solvent. Based on response surface and contour plots, was concluded that selective extraction of Ba (V) and αg soyasaponins were directly proportional with temperature and inversely proportional to pressure. These selective saponin extracts showed high potential to prevent and control hyperglycemia by inhibiting key enzymes in carbohydrate metabolism; proven power to counteract oxidative stress by protecting against free radicals, and high activity to inhibit inflammation, by regulating the production of cellular levels of nitric oxide. SC-CO_2 extraction is a feasible technology to obtain high-value compounds from by-products to treat metabolic syndrome.

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Supercritical CO_2 extraction as a tool to isolate phytochemicals from Rice (*Oryza sativa L.*) by-products

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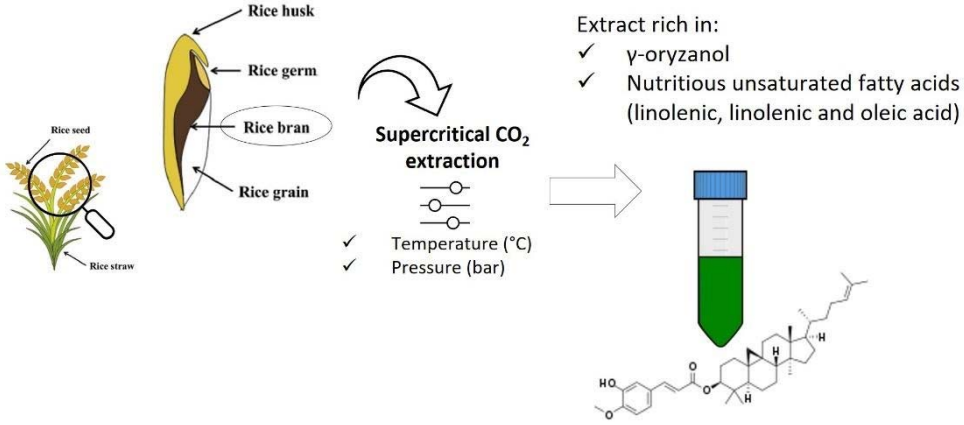
GRAPHICAL ABSTRACT



ABSTRACT

Rice bran is an important byproduct of rice milling industry with a global potential of 29.3 million tons annually. It is gaining great attention of the researchers due to its nutrient-rich composition, easy availability, low cost and promising effects against several metabolic disorders. Bioactive components of rice bran, mainly γ-oryzanol, have been reported to possess antioxidant, anti-inflammatory, hypocholesterolemic, anti-diabetic, and anti-cancer activities [1], [2]. Rice bran oil contains appreciable quantities of bioactive components (tocopherols and tocotrienols) and has attained the status of "Heart oil" due to its cardiac-friendly chemical profile [3], [4].

This work is part of TRACE-RICE | PRIMA project in which one of the main goals is to obtain innovative natural ingredients from rice (*Oryza sativa L.*) by-products. The aim is to significantly increase their potential value, pursuing nutritive and healthy targets using circular economy and sustainability approaches. Within this context, green extraction and

separation methodologies are being explored for the extraction and recovery of high valuable compounds from rice bran.

Rice bran of indica and japonica rice cultivars were processed by supercritical carbon dioxide to recover bioactives-rich fractions. The effect of different process parameters on the extraction performance (extraction yield and chemical composition) were evaluated and optimized, including operating temperature (40 – 80 °C) and pressure (200-500 bar), in order to maximize the extraction of high valued nutritional compounds (such as γ-oryzanol and fatty acids). Moreover, for comparison purposes conventional S-L extractions using combinations of n-hexane, ethanol and water as primary solvents were also performed. Extracts were analyzed by HPLC and LC-MS/MS. Total phenolic compounds were measured by *Folin Ciocalteu* method and antioxidant activity of the extracts were also evaluated by oxygen radical absorbance capacity assay (ORAC).

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Extraction from hemp biomass and seed

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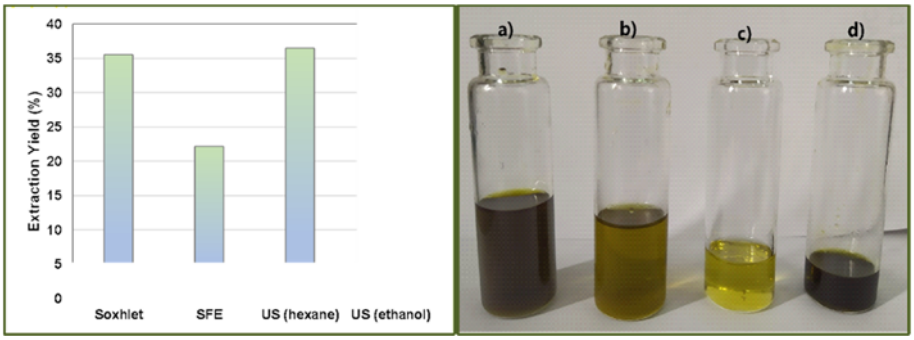
GRAPHICAL ABSTRACT



ABSTRACT

We carried out an exploratory study of the extraction with various techniques from hemp biomass and from hemp seeds. We compared extraction with supercritical CO_2, Soxhlet and ultrasound-assisted extraction. As solvents we chose ethanol for its low toxicity, being considered GRAS and hexane, because it is accepted and widely used in the food industry for vegetable oils recovery. For hemp biomass, we also used olive oil as the extract could be further used for the preparation of cosmetics and topicals.

The extraction yield, selectivity as a function of the concentration of the desired compound (CBD in crude extract from biomass and lipids+tocopherols+carotenoids in oil from seeds), and chlorophylls was compared. It was also measured the antioxidant capacity and analyzed the microbiological quality. In addition to, we used as comparative criteria to decide the best technology, the raw materials/energy demand for both extraction and solvent recovery, as well as the operating time and possibility of continuous operation because it directly affects the production capacity. For extracts, we qualitatively evaluated the need for post-processing and its complexity, as well as the end-product market. Safety and environmental aspects were also considered.

We will present the current state of extraction of this raw material in Spain.

ACKNOWLEDGEMENTS
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Supercritical fluid extraction and fractionation of the oil from marina microalgae *Crypthecodinium cohnii* to obtain biodiesel and omega-3 compounds (DHA)

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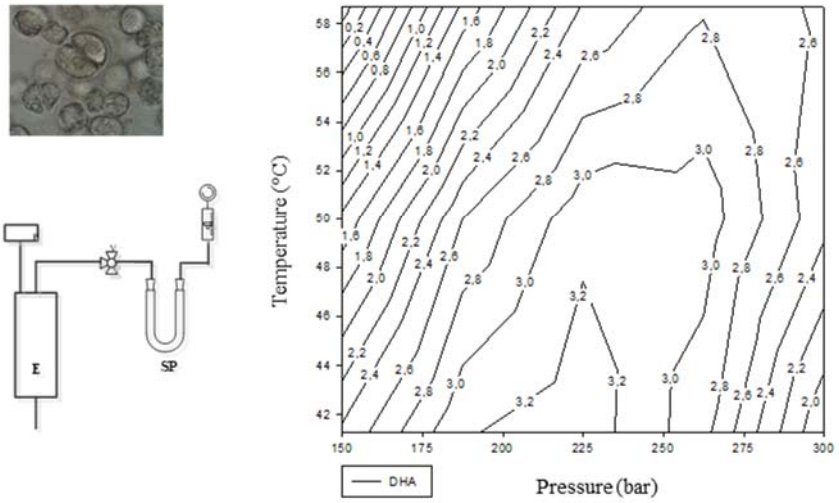
GRAPHICAL ABSTRACT



ABSTRACT

Oleaginous microalgae are considered a promising raw material for the production of biodiesel and ω-3 compounds, which have important applications in the food and pharmaceutical industry [1]. The oleaginous marine microalga *Chrypthecodinium cohnii* (*C. cohnii*) produces significant amount of lipids with a high content in docosahexanoic acid (DHA), a polyunsaturated ω-3 fatty acid (PUFA) with recognised benefits to human health, which is currently widely used in food and pharmaceutical formulations [2]. The remaining fractions of saturated and unsaturated lipids from this microalga can be employed to produce biodiesel, whose use reduces CO_2 emissions, as well as the greenhouse effect [1,2].

Microalga *C. cohnii* growth conditions have been studied and optimized at the Bioenergy and Biorefineries Unit of LNEG with the aim of obtaining high density cultures presenting a biomass rich in lipids, with a high lipid content on DHA [3].

The present work aimed to explore the use of supercritical carbon dioxide for the extraction and fractionation of lipids from the produced microalga biomass and to

implement a simple and environmentally friendly process for the co-production of biodiesel and the high value-added product, DHA. With the purpose of optimizing the experimental extraction conditions for the maximum yield, an experimental design based on a surface response methodology, according to the Doehlert distribution for two factors, was built [4,5]. The two studied factors were pressure (150-300 bar) and temperature (40-60 ºC), being the response factors the yield in lipids, in DHA and in fatty acids targeted to biodiesel production. It was found that pressure had a more pronounce effect than the temperature on the lipid extraction yield. Moreover, the isoresponse surfaces corresponding to the maximum yield in lipids were obtained in two distinct ranges of pressure and temperature: 187.5-240 bar/40-46 ºC and 250-300 bar/52-60 ºC. On the other hand, concerning the DHA yield, it was observed that both factors had a similar effect on the extraction of this compound. Moreover, the maximum value of DHA yield was obtained for the pressure/temperature ranges: 190-235 bar/ 40-47 ºC. Finally, the maximum yield in fatty acids for biodiesel was obtained in the lower right quadrant of the isoresponse surface graphic, corresponding to the ranges of lower temperature: 43-51 ºC and high pressure: 225-270 bar.

These results were taken into account to perform the fractionation of *C. cohnii* lipids for biodiesel and high value-added value DHA. The fractionation studies were carried out through a sequential extraction over time at 41.3 ºC and 187.5 bar. First faction was collected after 30 min extraction and the second fraction after 2.5 hours. It was possible to recover 21% of the total lipids in the first fraction with a 38% (w/w) content of fatty acids for biodiesel. On the other hand, 64% of the DHA in the biomass was recovered in the second fraction. Therefore, it was possible to obtain a first fraction with saturated and monounsaturated fatty acids targeted to biodiesel production, and a second fraction enriched in DHA.

ACKNOWLEDGEMENTS

This work was financed by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., within the scope of the project PTDC/EAM-AMB/30169/2017, titled "OMEGAFUEL – New platform for biofuels and omega-3 compounds production, from the marine microalga *Crypthecodinium cohnii* sustainable biorefinery". The authors also thank FCT for financial support through the funding UIDB/00100/2020 and UIDP/00100/2020. Beatriz P. Nobre thanks Instituto Superior Técnico for the Scientific Employment contract IST-ID/ 14/ 2018.

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Accessing cork biomass fractions via conventional chemical and high pressure and temperature water

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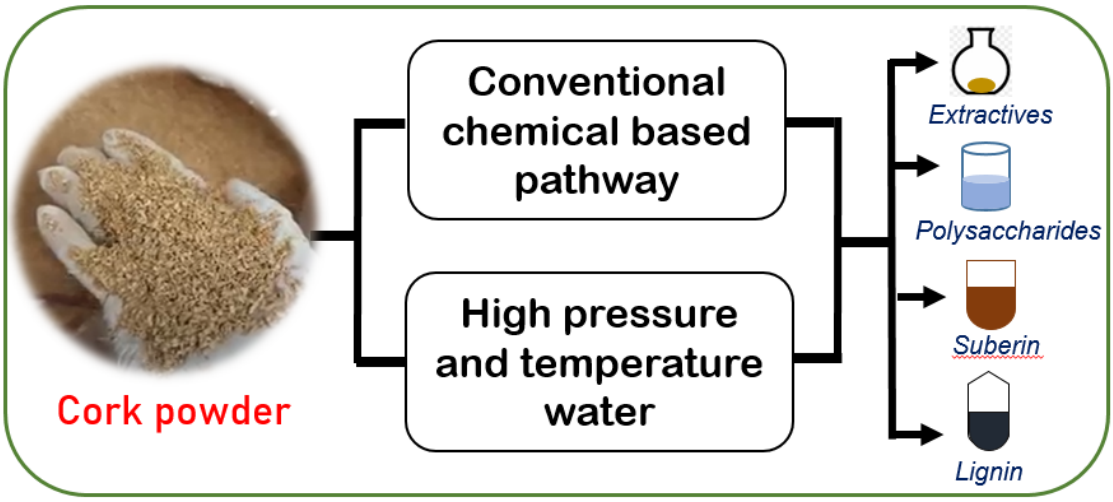
GRAPHICAL ABSTRACT



ABSTRACT

Lignocellulosic materials, which are found abundantly in agro-industrial wastes and residues can be an effective source of important molecules and building blocks that can be processed into bioenergy, biofuels, biochemicals and other value added products. One of the major issues that needs to be addressed in utilizing lignocellulosics is the dire need for an effective biomass fragmentation that would enable industrial application. It was reported that effective biomass fragmentation strategies should adhere to the following criteria to be industrially acceptable [1]:

i. it should, as much as possible, consist of the minimum number of steps;
ii. it should minimize if not completely avoid the use of organic chemicals and or harsh conditions, and
iii. ideally founded upon well recognized processes or practices in the chemical industry.

Biomass fractionation has been effectively carried out under supercritical water conditions operated under temperatures and pressures higher than the critical point of water (Tc = 647 K and Pc = 221 bar) [2]–[5] through hydrolysis reactions. Our group has developed the Sudden Expansion Micro-Reactor (SEMR) that enables ultrafast hydrolysis reactions with residence times as short as 40 ms. The SEMR has been recently demonstrated as an effective approach in valorizing actual lignocellulosic biomass, including wheat bran, sugar beet pulp, kraft lignin and grape seeds [6]–[9]. With the accumulation of learning about SEMR in various lignocellulosics, it is now envisioned to

work on more comples biomass. One biomass of complex structure is found in the bark tissue of *Quercus suber*, more commonly known as cork.

Cork is comprised of extractives, polysaccharides, suberin and lignin, all of which have distinct structures and promising applications [10], [11]. Given such heterogeneity and complexity, cork fractionation necessitated huge amount of organic chemicals as well as long processing times (that lasted between two-three days and involved at least nine steps in succession). The product yields were found to be in range of a few grams. Indeed, ultrafast hydrolysis with SEMR could then desirable to shorten the processing time with the minimum number of steps possible and avoid massive organic chemical utilization. Our preliminary experiments revealed that the ultrafast hydrolysis of cork can take place in as short as 0.25 s (395 °C, 265 bar). The thorough knowledge obtained in during chemical processing helped in identifying conditions for the SEMR experiments. A walkthrough of the processes involved in both chemical and SEMR approach, as well as the characterization of the individual components obtained will also be discussed during the presentation.

ACKNOWLEDGEMENTS
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Subcritical water extraction of quercetin and derivatives from onion skin wastes (*Allium cepa* cv. Horcal): effect of temperature and solvent properties

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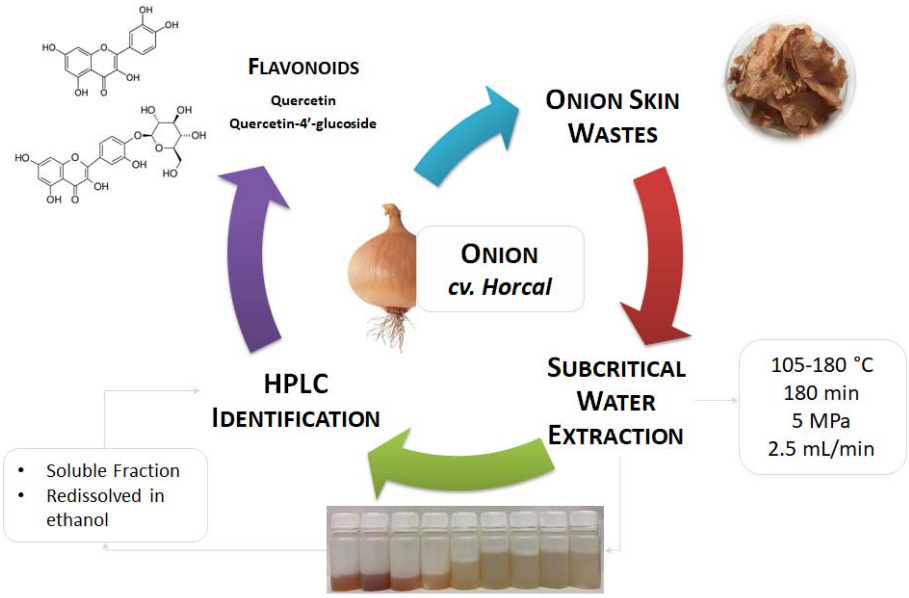
GRAPHICAL ABSTRACT



ABSTRACT

Nowadays, the society is immersed in the transition from a linear to a circular economy, in which the value of products, materials and resources is maintained in the economy for as long as possible and the generation of waste is minimized. This means that the byproduct of a process becomes the input of a new one where it acquires new value. Among other agricultural by-products, onion (*Allium cepa* L.) skin wastes offer great potential for valorization. Onion is the second most important horticultural crop worldwide. More specifically, Spain, in the year 2018 produced 1.27 Mt [1]. The onion industry generates every year more than 0.5 Mt of Onion Skin Wastes (OSW) worldwide [2], including skins (the outermost layers), roots and bulbs unfit for consumption. The non-edible brown skin and external layers of onions are rich in phenolic compounds, mainly flavonoids such as quercetin (QC) [3] and its derivatives: quercetin 4'-O-β-glycoside (QC4'), quercetin 3,4'-O-β-diglycoside (QC3,4') and quercetin 3-O-β-glycoside (QC3). All of them are high-added value natural antioxidants [2]. The main drawback of quercetin and quercetin derivatives is the limited solubility in water, which limits their oral bioavailability [4] and extractability, and forces the use of an organic solvent to successfully extract them. Alternatively, Subcritical Water (SubW) can be used

to extract flavonoids from onion skins. SubW refers to water at temperatures ranging from 100 °C (boiling point) to 374 °C (critical point) which remains in a liquid state due to the application of pressure. Changes in the working conditions change the properties of the SubW (among them, viscosity, surface tension and dielectric constant, which can be similar to those of some organic solvents), enhancing mass transfer and the extractability of barely water-soluble bioactive compounds, as summarized by Benito-Roman et al. [5], as SubW favors the hydrolysis of the bonds between phenolic compounds and the vegetable matrix.

In this work the extraction, identification and quantification of phenolic compounds from OSW has been studied using SubW in a semicontinuous extractor (flow rate constant and equal to 2.5 mL/min; temperatures up to 180 °C with working pressure of 5 MPa, to keep water in liquid state). The extraction of flavonoids resulted to be fast (<30 min) and temperature sensitive (maximum at 145 °C; total flavonoids, 27.4±0.9 mg/g OSW). Further increases of temperature decreased the number of flavonoids recovered. The experimental results were fitted to the Weibull model. The influence of the solvent properties on the flavonoids quantification was found to be critical. A precipitate was formed once the extracts cooled down. If removed, a significant fraction of the high temperature extracted flavonoids (as much as 71%, at 180 °C) was lost. This fact affected especially to those compounds that show extremely low solubility in water at room temperature, whereas quercetin glycosylated derivatives were less affected by the polarity change of the medium induced by the temperature change. It has been demonstrated that it is necessary to re-dissolve the subcritical water extracts by the addition of ethanol, which led to a medium with a polarity equivalent to that obtained with water at high temperature. At 145 °C, quercetin (15.4±0.4 mg/g OSW) and quercetin-4'-glucoside (8.4±0.1 mg/g OSW) accounted for the 90% of the total flavonoids identified by HPLC, according to the method described by Benito-Román et al. [6]. All in all, the obtained extracts resulted to be rich in flavonoids, which makes them suitable to be used as food additives in order to replace other synthetic antioxidant compounds.

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Novel micelles of PEG functionalized with coumarin toward multifunctional drug delivery systems

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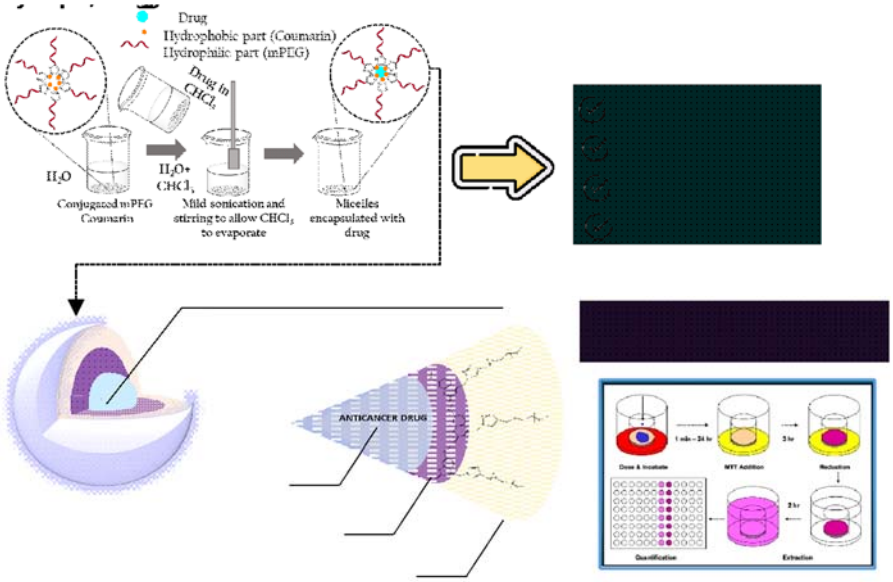
GRAPHICAL ABSTRACT



ABSTRACT

Chemotherapeutic agents are typically water-insoluble, and their therapeutic effect is compromised by the short circulation time and systemic toxicity [1]. Recently, nanocarriers have generated a huge interest in the field of research in cancer therapy due to the numerous aspects which have a remarkable influence on the efficacy of treatments [2]. Among the widely classification of polymeric nanocarriers, the polymeric micelles are widely studied molecules in drug delivery because of their biocompatibility, low toxicity and high stability. The development of polymer drug conjugates (PDCs) is an important and simple strategy to design the self-assembly of amphiphilic conjugates for micelle preparation [3].

In this study, a PDC was synthesized using polyethylene glycol (PEG) as hydrophilic segment and a natural drug, coumarin, as hydrophobic one. Fabrication of PEG-drug conjugates is an important and facile strategy to prepare nanomedicines by the self-assembly of amphiphilic conjugate. The methodology used in this work was click chemistry. Click chemistry has been used in the synthesis of polymers with pharmaceutical, biomedical applications and modification of nanoparticles. Combined

with the use of supercritical CO_2 ($scCO_2$), it will protect drugs from degradation and avoid the use of toxic solvents. A study on the behaviour of PEG in $scCO_2$ was carried out [4], different copper catalysts were and operating conditions was optimised in $scCO_2$ [5]. Once the conjugated coumarin-PEG (CouPEG) reaction in $scCO_2$ was achieved, the next step was the micelles preparation. CouPEG could self-assembled into micelle to encapsulate paclitaxel (PTX), Gemcitabine (GEM) and Curcumin (CUR). Subsequently, the self-assembly behaviours, drug loading capacities and different entrapment drugs were carefully studied. The next steps, an *in vitro* release assay was carried out and mathematical modelling was applied. Finally a preliminary study of CouPEG micelles *in vitro* cytotoxicity was carried out with PANC-1 and bxPC-3 cells.

ACKNOWLEDGEMENTS

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Soybean oil-based cyclic carbonates synthesis in supercritical CO_2 sustained by halide organocatalysts.

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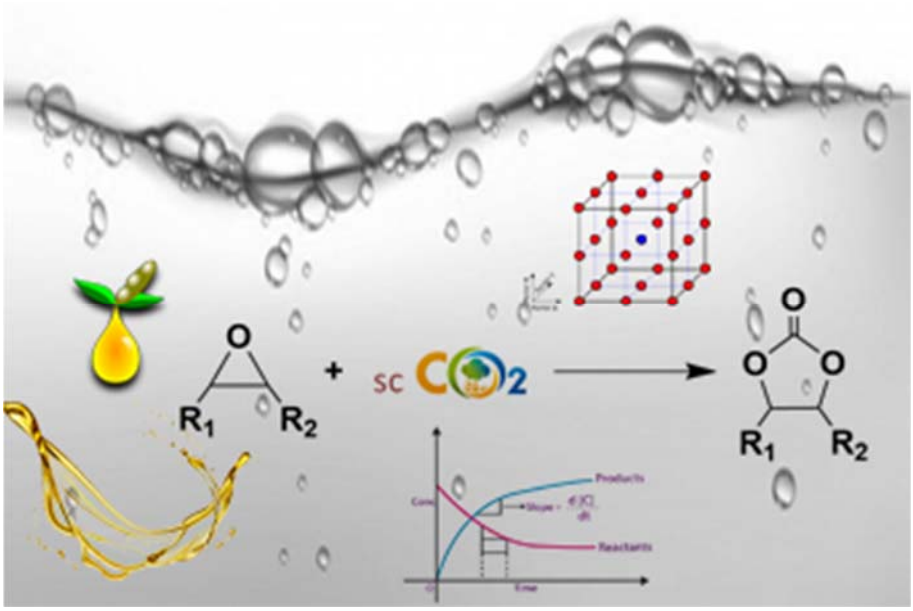
GRAPHICAL ABSTRACT



ABSTRACT

Carbon dioxide (CO_2) utilization as feedstock for chemical derivatives is one of the most promising alternatives to replace fossil sources as well as to mitigate global warming effects. At the same time, sustainable biomass resources demand experiment a constant growth, especially those like vegetable oils, which, apart from its renewable origin and biodegradability, provides high functionality and several possibilities as a the structural backbone of different chemical products and materials like biodiesel, polyesters, epoxies or non-isocyanate polyurethanes (NIPUs) [1,2].

Precisely the combination of both routes allows the exploration of procedures of great interest, such as the incorporation of CO_2 in bio-based epoxides to obtain cyclic carbonates, the main precursor of the previously mentioned NIPUs. [3].

Supercritical conditions are barely investigated in literature [4] as the selected media for the synthesis of this carbonates, despite its high potential to increase the reaction yields, as a result of its unique properties that allow to overcome mass transfer limitations.

Therefore, this research is focused on the study of the kinetics of the process of incorporation of supercritical CO_2 into the molecular structure of epoxidized soybean oil, using previously demonstrated high efficiency organocatalysts. Finally, an optimization of the operating conditions is carried out, having a future industrialization vision of the process in mind.

ACKNOWLEDGEMENTS
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HIGH PRESSURE PHASE EQUILIBRIA PREDICTION OF RENEWABLE LIGNING DERIVED AROMATIC COMPOUNDS WITH CO_2

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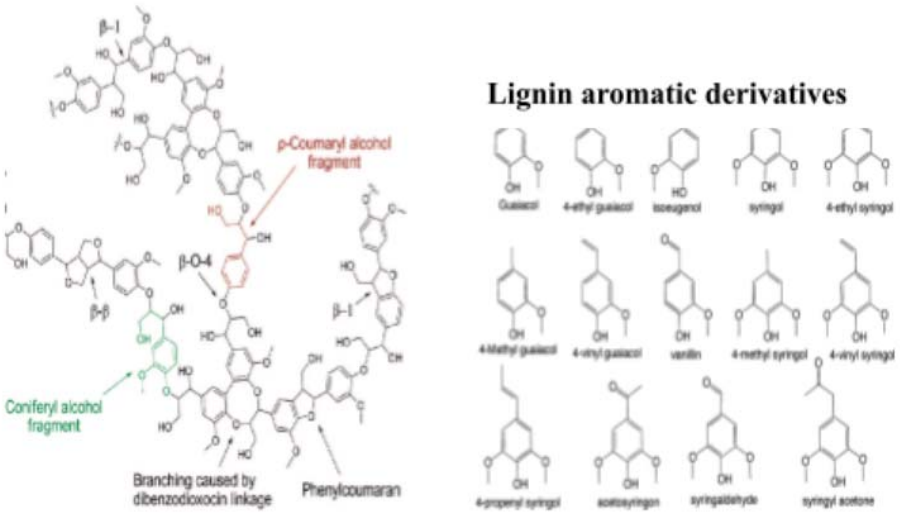
GRAPHICAL ABSTRACT



ABSTRACT

Biomass fractionation into its individual building blocks is a major challenge, in particular, for the valorization of low-cost raw biomass or subproducts/residues from the biomass processing industry, like gums or kraft lignin from oil and pulp plants. Aromatic rings are present in biomass as part of lignin structure and as linking compounds of carbohydrate polymers. There are several pathways to fractionate these natural polymers, among thermal treatments pyrolysis and hydrolysis are both relevant, also base catalyzed depolymerization or oxidative cleavage [1]. In fast pyrolysis, the bio-oil is a promising alternative to fossil fuels and is currently entering the heating oil market. [dahmen] In addition, it can be further processed to synthesis gas, from which synthetic fuels and chemicals can be obtained. Nonetheless, to boost the whole process economy, it is important to recover as much valuable compounds as possible, prior to either burning or gasifying the bio-oil. Similarly, lignin hydrolysates also comprise aromatic compounds

to be recovered from the pre-treatment product. In summary, bio-oils refining is calling for research in separation technologies, which are key to trigger the development of biomass fractionation processes. In particular, related to high pressure technology in this field, early works apply CO_2 extraction as a non-destructive solvent free analytical method for biooil characterization [3,4] and, more recently, several reviews consider CO_2 extraction as a feasible technology for scaled-up recovery of valuable compounds [XX]

As it is well known, to design and optimize separation processes, it is essential to have a robust thermodynamic model, able to predict the phase equilibria of bio-oils. Regardless of the upstream biomass depolymerization technology, there are common features that bio-oils from different sources share: 1) multicomponent asymmetric mixtures comprising a high number of compounds, 2) non-ideal behavior due to the presence of water and organo-oxygenated compounds, 3) comprise multifunctional molecules whose properties are in general unknown, 4) presence of solid precipitates. The phase behavior of biomass derived mixtures is highly non-ideal due to the presence of association and solvation effects. The numerous oxygenated derived species belong to certain families of organic compounds (alcohols, carboxylic acids, esters, etc) thus these complex mixtures with hundreds of compounds can be described by a reduced number of functional groups. For this reason, the use of a group contribution approach is a logical choice for thermodynamic modeling.

The Group Contribution with Association Equation of State (GCA-EoS) is especially suited for this aim [6]. It has already proved excellent predictive capacity to represent the phase behavior of complex mixtures containing natural products and biofuels. As part of this work, GCA-EoS is extended to describe phase behavior of aromatic lignin derived compounds based on low pressure phase behavior data of simple monofunctional aromatic compounds and their binary mixtures with CO_2 available in the open literature. Once the aromatic functional groups are parametrized, the GCA-EOS will be challenged to predict high pressure phase behavior of multifunctional aromatic lignin derived compounds in mixtures with CO_2.

ACKNOWLEDGEMENTS

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Machine learning models for the prediction of diffusivities in supercritical CO_2 systems

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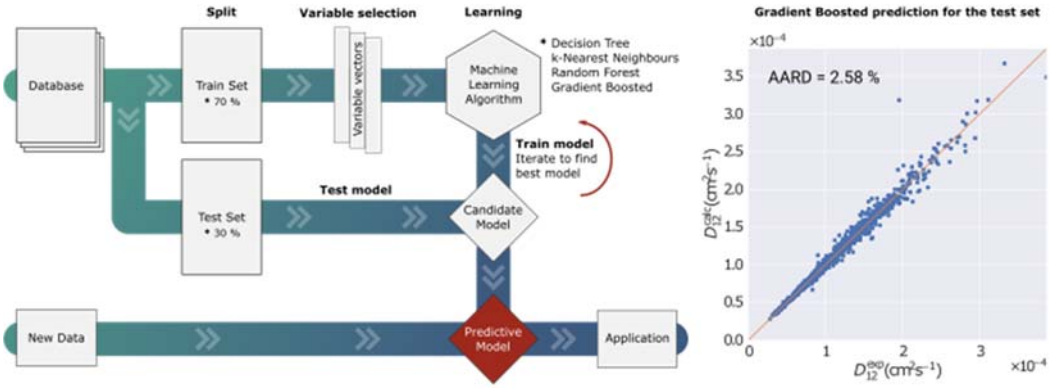
GRAPHICAL ABSTRACT



ABSTRACT

Transport properties are extremely important for the design, simulation and scale-up of separations and chemical reactions. One such property is the molecular diffusion coefficient, D_{12}, which is fundamental to estimate dispersion coefficients, convective mass transfer coefficients, catalysts efficiency factors, etc. [1]. Supercritical carbon dioxide (SC-CO_2) is one of the so-called "green-solvents", which have been gaining traction in both academia and industry. SC-CO_2 has been extensively used in the supercritical extraction (SFE) of compounds from multiple vegetable matrices [2]. However, experimental diffusivity data in SC-CO_2 is still scarce in terms of solutes and operating conditions, requiring accurate models capable of providing reliable D_{12} estimations.

Currently, the Wilke-Chang equation [3], which is a modification of the Stokes–Einstein equation, is the most well-known and most used equation to calculate solute diffusivities in SC-CO_2. Other models have been proposed, such as the Lai-Tan equation, a modification of the Wilke-Chang equation specifically devised for SC-CO_2, and the correlation of Dymond-Hildebrand-Batschinski (DHB), which given some known data about the system allows for interpolation and extrapolation to the desired conditions [4].

In this work, we developed predictive models to estimate diffusivities of solutes in supercritical carbon dioxide, using machine learning models. A large database of experimental data containing 21 properties for 174 solutes and 4917 data points (covering small and large, polar and nonpolar solute molecules) was used in the training of the machine learning models. The database was randomly split 70/30 % into training and testing sets, respectively. Four machine learning models were evaluated: a k-Nearest

Neighbors model, a Decision Tree algorithm, and two Ensemble Methods (Random Forest and Gradient Boosted). The results were compared with a simple multi-linear regression and with the conventional models of Wilke-Chang, Lai-Tan, and DHB [4].

The best results were found using the Gradient Boosted algorithm which showed an average absolute relative deviation (AARD) of 2.58 % (see Figure) for the 1476 points in the test set (points not used in model training). This model has six parameters including temperature, pressure, density, solute molar mass, solute critical pressure, and solute acentric factor. The k-Nearest Neighbors, Decision Tree and Random Forest models presented overall results between 4.1 % and 5.5 %. By comparison the multi-linear regression obtained an AARD of 15.86 %. The conventional models of Wilke-Chang, Lai-Tan, and DHB, showed worse performance for the same test set with deviations of 12.41 %, 26.01 % and 4.27 %, respectively [4]. Although the DHB model shows performance similar to the machine models, it requires previous experimental data to fit the system parameters, which is not always possible. A simple-to-use command line application was created to allow users to apply the Gradient Boosted model developed here to the prediction of diffusivities in SC-CO_2.

ACKNOWLEDGEMENTS

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Batch and continuous production of directly compressible drug nanopowders using CO_2-assisted spray coating

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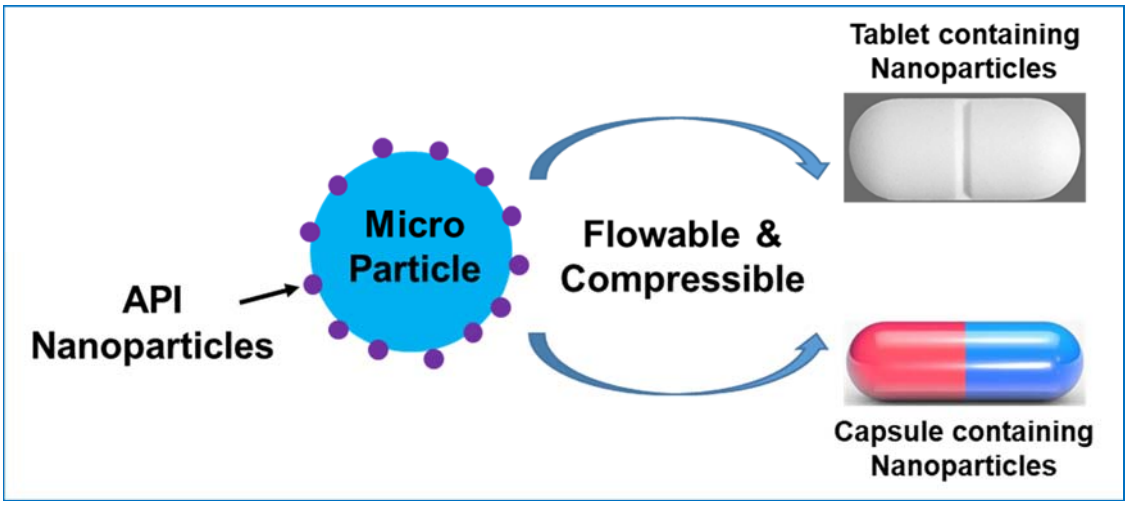
GRAPHICAL ABSTRACT



ABSTRACT

Poor solubility of new Active Pharmaceutical Ingredients (APIs) is a major bottleneck in the pharmaceutical industry which typically leads to poor drug bioavailability and efficacy. Nanotechnologies offer an interesting route to improve the apparent solubility and dissolution rate of pharmaceutical drugs, and processes such as nano-spray drying provide a route to engineer and produce solid drug nanoparticles [1-2]. However, dried nanoparticles often show poor rheological properties (e.g. flowability, tabletability) and their isolation using these methods is typically inefficient and leads to poor collection yields [3-4]. Indeed, nanoparticles can exhibit poor flowability and compressibility due to their charged nature thereby driving them towards agglomeration or crystal growth. This leads to an inefficient drug formulation and hence drug failure during the clinical stages. Hence, there is a great demand in the pharmaceutical sector to isolate drug nanoparticles by uniformly capturing/coating them onto other micron-sized carrier particles, or encapsulating them in polymer matrices, thereby, improving rheological behaviour in drug product.

The work presented demonstrates novel processes (batch vs continuous nano-spray coating) for the single-step production and isolation of pharmaceutical drug nanoparticles, which use supercritical CO_2 as the atomizing and fluidizing gas [5]. Nanoparticles of BCS class II and IV APIs, namely carbamazepine (CBZ), ketoprofen (KET) risperidone (RIS), Celecoxib (CEL) and Hydrochlorothiazide (HTZ) were produced and successfully coated onto micron-sized microcrystalline cellulose (MCC)

particles (10% and 20% drug loadings), and their physicochemical properties were analyzed.

The batch and continuous nano-spray coating processes developed provide single-step approaches to produce and isolate (with collection yields up to ~90%) solid nanoparticles with optimal dissolution and rheological properties. Powder X-ray diffraction (PXRD) analysis confirmed the production of crystalline nanoparticles which were coated onto MCC particles with stable polymorphic forms for CBZ, KET, CEL and HTZ, while metastable form was obtained for RIS. The isolated API-MCC composite samples presented optimal rheological properties which were confirmed from the compressibility data analysis. Further, a 6-15 fold increase in the dissolution rate of CEL nanoparticles was observed, while a 6–7 fold increase was observed for RIS nanoparticles, a 3–4 fold increase for CBZ nanoparticles, a 2–3 fold increase for KET nanoparticles and a 2.5 fold increase for HTZ nanoparticles.

ACKNOWLEDGEMENTS
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Micronization of Ciprofloxacin by the Supercritical Antisolvent Technique (SAS)

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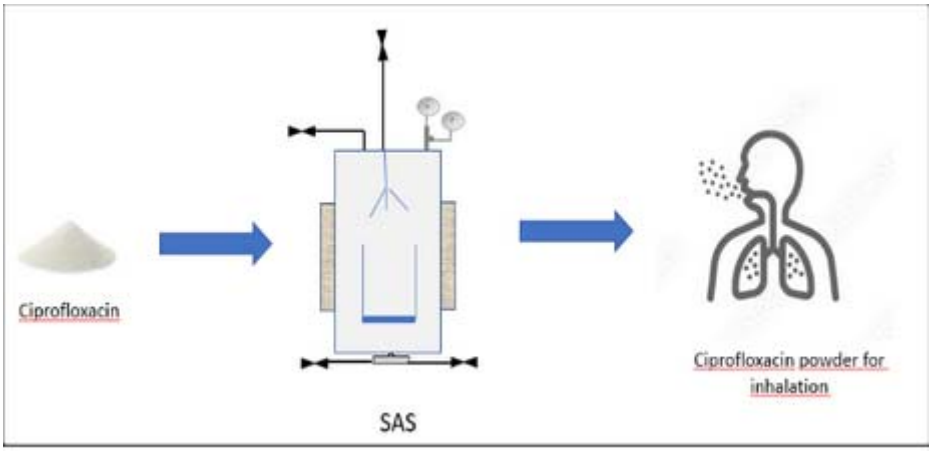
GRAPHICAL ABSTRACT



ABSTRACT

Ciprofloxacin is an antibiotic that belongs to the fluoroquinolone family. It has a broad antibacterial spectrum and is currently used to treat many infections [1]. The pharmacological characteristic of ciprofloxacin makes it considered a suitable drug for administration by inhalation to achieve high concentrations of the drug in the lung [2].

The recommended particle size for administration by inhalation is less than 5 μm, because this size allows a deeper distribution in the airways, which will cause a better therapeutic response [3]. Thus micronization of the drug is required. Micronization of drugs allows modifying their pharmacokinetic and pharmacodynamic properties, by improving their bioavailability, their solubility and consequently reducing their adverse effects.

Supercritical fluids have been extensively used in the precipitation/micronization of drugs. CO_2 is the most common supercritical fluid because of its mild critical conditions, 31.1°C and 7.38 MPa, making it suitable for processing heat-sensitive compounds. Furthermore, it is cheap, non-flammable, non-toxic, inert, chemically stable, and it has GRAS status. Being a gas under normal conditions, it can be eliminated by lowering the pressure and can be recycled.

The solubility of Ciprofloxacin in supercritical CO_2 has been measured and it is very low, therefore among the different micronization techniques, the Supercritical Anti-Solvent (SAS) technique was used.

Ciprofloxacin is soluble in dilute hydrochloric acid and acetic acid but practically insoluble in ethanol [4]. However, its solubility in ethanol can be greatly improved by the addition of a small amount of acetic acid.

In this project, the micronization of a solution of Ciprofloxacin in ethanol with 2% v/v acetic acid by the SAS technique was attempted. The apparatus and experimental procedure have been previously described in detailed [5].

Briefly, ca. 35 ml of the ciprofloxacin solution was injected into the precipitation chamber through a 100 μm stainless steel nozzle at a flow rate of 1.5 mL/min forming droplets. At the same time, supercritical CO_2 was pumped into the chamber at 20 g/min. The droplets mixed with supercritical CO_2 inside the precipitation chamber, leading to the expansion of the ethanolic solution, its supersaturation and the precipitation of the drug. The precipitated particles were kept in the chamber using a 0.2 μm nylon filter over the 2 μm steel frit set at the bottom of the chamber. CO_2 and the depleted solution flowed through the BPR and were separated in a cyclone separator at atmospheric pressure. After the solution pump was stopped, 1.5 L of CO_2 (three times the volume of the chamber at the chosen precipitation conditions) were allowed to flow through the chamber to dry and completely remove the solvent from the precipitated solute.

The precipitate was characterised using FTIR and DSC techniques, showing no significant differences when compared to a Ciprofloxacin base standard, confirming that the chemical structure of the drug was not altered. SEM micrographs showed elongated particles of submicron width and up to 5 μm long, demonstrating the successful micronization of the drug. Great changes in the morphology and size of the drug were observed with the precipitation conditions.

Antibacterial activity of the precipitated ciprofloxacin was evaluated against standard laboratory strains of *Staphylococcus epidermidis* (CECT 232), compared to a standard Ciprofloxacin base and the results obtained show that the antimicrobial activity of the drug is maintained after the precipitation process.

ACKNOWLEDGEMENTS

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Manufacturing of liposomal-loaded hydrophilic or hydrophobic drugs dry powder formulations using supercritical carbon dioxide

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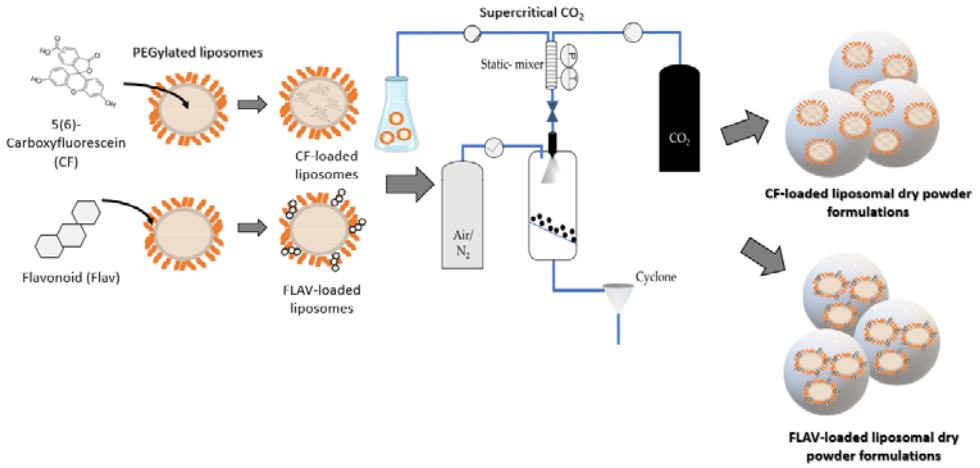
GRAPHICAL ABSTRACT



ABSTRACT

Chronic obstructive pulmonary disease, asthma and cystic fibrosis are the most common chronic neutrophilic inflammatory lung diseases, requiring high doses of anti-inflammatory drugs. Unfortunately, a long-term systemic therapy with the common medicines as corticosteroids can provoke adverse effects such as osteoporosis, adrenal suppression, diabetes, and cardiovascular diseases [1]. Biopharmaceuticals and flavonoids with anti-inflammatory properties can overcome those drawbacks and provide a healthier patient treatment. After parenteral administration, PEGylated liposomes, owing to their physical and chemical features, can carry hydrophilic and/or hydrophobic drugs, like biopharmaceuticals and flavonoids, respectively, circulating in the bloodstream up to 24 h [2]. Yet, due to their limited stability in suspension upon storage that might limit their use, it is advantageous to convert them into solid dosage forms. Besides, in powder form, liposomes can be administered to the lungs. Therefore, using a

green technology like supercritical CO_2-assisted spray-drying (SASD), liposomal suspension is converted into dry powder formulations with capable of administration via different routes, including the lungs. In this work, liposomes, encapsulating 5(6)-carboxyfluorescein (CF) as a marker of the internal aqueous phase, were produced and then dried using the SASD [3]. After resuspension in water to remove the excipient, CF encapsulation efficiency remained above 95 %. In parallel, a flavonoid (FLAV) was incorporated into liposomes. Powders showed a mass median aerodynamic diameter and a fine particle fraction suitable to be inhaled. The CF-loaded liposomal dry powder formulations were submitted to storage stability assays at relative humidity of 4 %, 50 % and 78 % for 30 days. Results showed that the dry powder formulations were able to maintain liposome stability at relative humidity of 4 % and 50 % at 20 °C for 30 days. This is a promising work targeted to the inhalable treatment of chronic pulmonary diseases with bioactive agents.

ACKNOWLEDGEMENTS

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Supercritical CO_2 technology for the production of sterile and drug-loaded scaffolds

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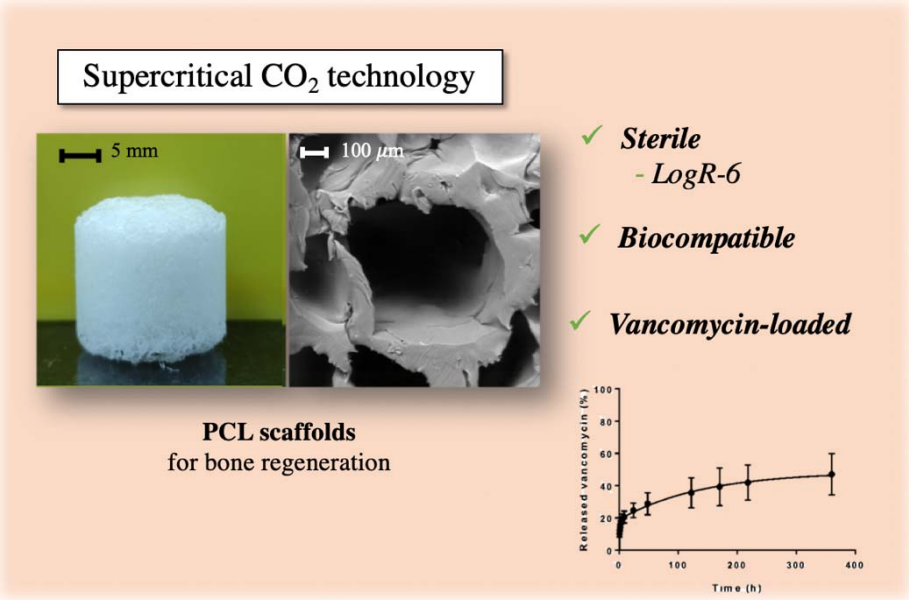
GRAPHICAL ABSTRACT



ABSTRACT

The procedure used for the sterilization of medical devices must ensure 6-logarithmic reductions (logR-6) against the most resistant microorganism towards the sterilization treatment, the so-called biological indicators (BI). Conventional sterilization techniques frequently lead to significant morphological and physicochemical modifications in the treated materials, jeopardizing the development and commercialization of new biomedical products, including polymeric scaffolds [1].

Supercritical CO_2 (sc-) incorporating low contents of hydrogen peroxide can inactivate bacterial endospores while preserving the physicochemical properties of the treated biomaterial, emerging as a promising novel methodology for scaffold sterilization. Recently, the spores of *Bacillus pumilus* were proposed as the BI to assess the sterilization efficacy of $scCO_2$-based methods [2]. On the other hand, $scCO_2$ can be exploited for the production of customizable drug-loaded scaffolds in the absence of solvents [3]. By the

fine control of the operating parameters, the scaffold porous architecture can be modulated in order to match the natural bone tissue properties [4].

In this work, the integration of the sterilization and foaming procedures was carried out for drug-loaded scaffolds, tackling one of the main ongoing technological challenges in the field. Vancomycin-loaded poly(ε-caprolactone) (PCL) scaffolds were obtained through a simultaneous sterilization and foaming procedure based on $scCO_2$ technology. PCL and vancomycin powders were poured in Teflon moulds and manually mixed before being subjected to the supercritical processing. Mild pressure and temperature conditions along with the addition of H_2O_2 (1200 ppm) ensured a logR-6 level against dry bacterial endospores of biological indicators (*Bacillus stearothermophilus*, *B. pumilus* and *Bacillus atrophaeus*). Finally, the potential extension of the supercritical sterilization process for the treatment of multicomponent medical devices was evaluated.

ACKNOWLEDGEMENTS

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Development of bioMIPs Using Supercritical Fluids

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GRAPHICAL ABSTRACT

ABSTRACT

Supercritical fluid technology provides a clean and straightforward way for the preparation of high affinity polymeric materials [1]. Affinity materials by taking advantage of the combination of Molecular Imprinting Technique (MIT) with supercritical dioxide carbon ($scCO_2$) have been developed for several applications, such as purification of Active Pharmaceutical Ingredients (API), removal of contaminants from water, and on-off sensors [1,2]. CO_2 is an abundant, non-toxic, non-flammable and inert gas with a very accessible critical point, which is easily removed in the polymerization step without additional energy input [3]. Molecularly Imprinted Polymers (MIPs) have been produced in a one-step synthetic supercritical route yielding molecular recognition materials, including biorecognition [4]. The use of MIPs for biomolecular recognition presents significant advantages when compared to natural antibodies [5], since they are robust, stable under harsh conditions of pH and temperature, without losing affinity, can be easily stored with a long lifetime, and thus high potential to overcome the drawbacks of antibodies in biorecognition processes. In addition, MIPs are cost-effective since high affinity is obtained by commercially and low-cost starting materials by using this sustainable technology [1]. Herein, bioMIPs were developed through a dual-template MIT approach of small biomolecules using $scCO_2$ technology. BioMIPs were obtained as dry, and free-flowing powders. The preliminary binding results are quite promising, revealing a cost-effective way to obtain quite stable polymeric materials for a wide range of biorecognition applications, and attractive for industrial applications.

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Customized and bioactive bone scaffolds obtained by a novel technological duo

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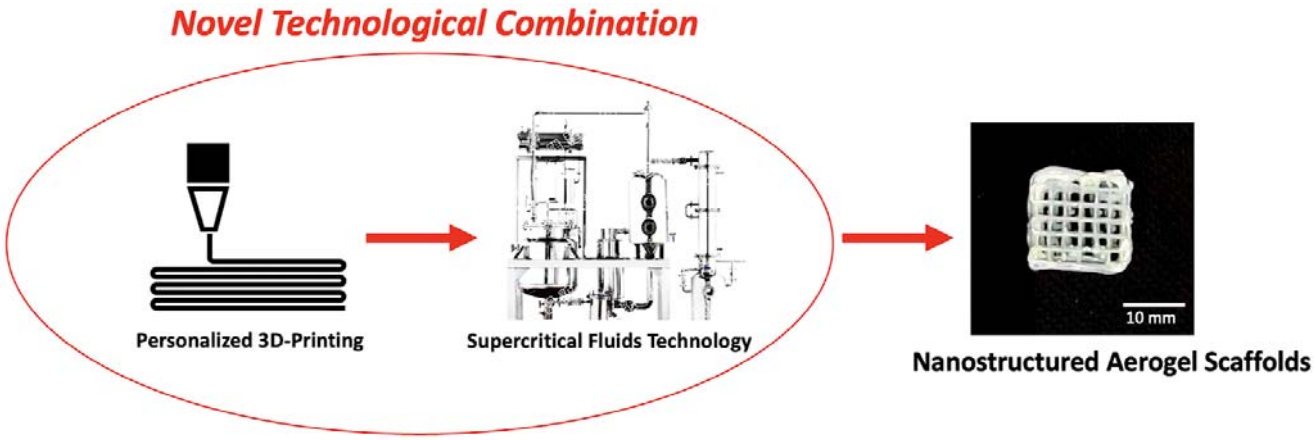
GRAPHICAL ABSTRACT



ABSTRACT

Bone is the second largest transplanted tissue worldwide with more than two million of cases taking place every year. The reconstruction of bone defects presents limited surgical options (mainly biological and synthetic implants). Good clinical results are achieved with the traditional surgical treatments, but scarcity or low osseointegration are only some of their drawbacks. Regenerative medicine seeks the complete anatomically and functionally restoration of a damage area by employing a provisional biodegradable support (also known as scaffold), cells and growth factors (1).

3D-printing is a disrupting technology that allows the manufacture of scaffolds with personalized and complex external morphologies and compositions (2). The reproducibility, scalability, high precision, and functionality of the 3D-printing method turn it into a suitable candidate for personalized medicine. Nevertheless, 3D-printed scaffolds usually lack a simultaneous control in the macro, micro and nanostructural levels. For this reason, the production of 3D-scaffolds composed by biocompatible materials with a well-defined external and internal structure is still a challenge in bone tissue engineering. Supercritical fluid technology based on the use of supercritical carbon dioxide ($scCO_2$) is a process able to preserve the polymeric gel structure on the dry state in terms of its textural and biological performance (3).

In this work, the novel technological combination of 3D-printing and supercritical fluid technology is used to obtain customized aerogel scaffolds with dual internal structure (mesoporous and macroporous) for personalized bone tissue engineering (3). Firstly, a biocompatible ink composed of alginate and hydroxyapatite (HA) is employed to generate customized gels with attractive structural and biological properties by 3D-printing. Then, the gel solvent is extracted by supercritical CO_2 drying, preserving its nanostructure and advanced physicochemical properties. BET and SEM analyses were performed to unveil the effect of the ink composition on the aerogel scaffold textural properties (specific surface area, porosity, mean pore diameter). Bioactivity, bio- and hemocompatibility were also confirmed by evaluating the biological behavior of the composites by SEM, WST-1 and hemolysis tests, respectively. Overall, this technological approach provides future perspectives towards the development of bone tissue engineering implants able to fulfill patient specific demands.

ACKNOWLEDGEMENTS

Work supported by MICINN [PID2020-120010RB-I00], Xunta de Galicia [ED431C 2020/17], Agencia Estatal de Investigación [AEI] and FEDER funds. Work carried out in the framework of the COST Action CA18125 "Advanced Engineering and Research of aeroGels for Environment and Life Sciences" (AERoGELS), funded by the European Commission. A.I.-M. acknowledges to Xunta de Galicia for her predoctoral research fellowship [ED481A- 2020/104].

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Short Oral Sessions

Combined supercritical fluid technology for the preparation of dry powders loaded with beclomethasone dipropionate for pulmonary delivery

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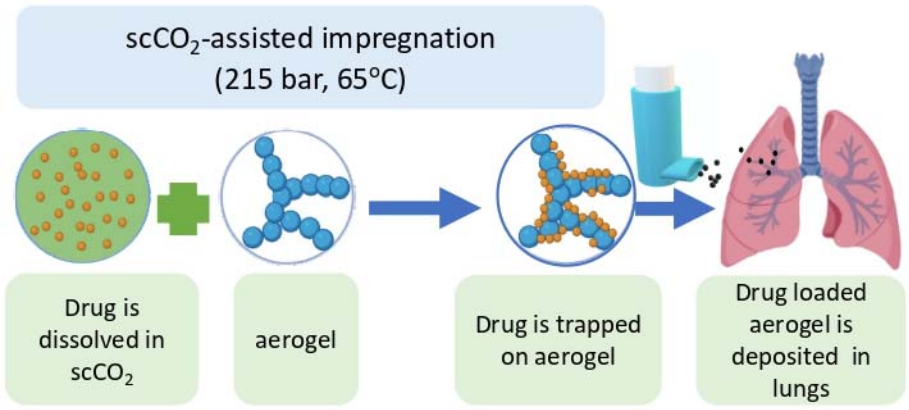
GRAPHICAL ABSTRACT



ABSTRACT

Novel strategies by pulmonary drug delivery are being developed for local or systemic treatments where the drug is targeted directly into the lung or entered into the bloodstream via the alveolar epithelium [1]. Among inhalation therapy, dry powder inhalers (DPIs) are inhaler devices particularly convenient for patients proving due to their portability, low cost and environmentally-friendly disposable and solid form. Various powder technologies have been used to produce DPIs, including jet milling and wet milling, spray drying or supercritical fluid drying (SCF) [1]. Among them, supercritical (sc) CO_2 has proved as an alternative powder technology that overcomes the challenge of conventional techniques, including cost-effective, non-toxic and able to modify the solid-state form of dry powder.

The combination of SCF and aerogels, the lightest material in the world, has recently received attention for its application in pulmonary drug delivery [2]. $ScCO_2$ drying is commonly applied to protect the structure of aerogels under mild conditions. Besides that, supercritical fluid CO_2-assisted impregnation (SCF CO_2-assisted impregnation) based SCF technology can be applied to incorporate drugs into the network of aerogels [3]. In

this context, $scCO_2$ will behavior as a solution for the loading of drugs in aerogel matrices. Unfortunately, this phenomenon may not favor for drugs that are poorly soluble in $scCO_2$.

In this work, beclomethasone dipropionate (BDP) loaded alginate aerogels were produced by combining supercritical fluid drying and impregnation technologies. BDP is an anti-inflammatory drug belonging to BCS-class II and commonly prescribed for the treatment of asthma. BDP-loaded alginate aerogels were obtained by emulsion-gelation methods following $scCO_2$ drying and impregnation. Due to the low solubility of BDP in $scCO_2$, various parameters were studied to optimize the BDP loading into the aerogel. These parameters include the impregnation time (2, 6 and 9 h) and the addition of co-solvents (methanol, ethanol and acetone). BDP-loaded alginate aerogels were tested regarding their morphological and textural properties by SEM and nitrogen adsorption-desorption analysis, the drug loading by HPLC and *in vitro* aerodynamic deposition with NGI impactor tests. The obtained inhaled particles have low density, nanostructures with open pores and high specific surface area. The use of co-solvents resulted in a significant increase in the BDP loading. Finally, the inhaled particles had low cohesive forces and high flowability according to the aerodynamic tests, resulting in a promising formulation for pulmonary drug delivery.

ACKNOWLEDGEMENTS

Work supported by MICINN [PID2020-120010RB-I00], Xunta de Galicia [ED431C 2020/17], Agencia Estatal de Investigación [AEI] and FEDER funds. Th. D. acknowledges COST Action CA18125 "Advanced Engineering and Research of aeroGels for Environment and Life Sciences" (AERoGELS), funded by the European Commission, for the granted Short Term Scientific Mission to evaluate the aerodynamic properties of the particles in the University of Parma.

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Supercritical fluid extraction of lupane triterpenoids from *Acacia dealbata* biomass – effect of pressure, temperature and cosolvents

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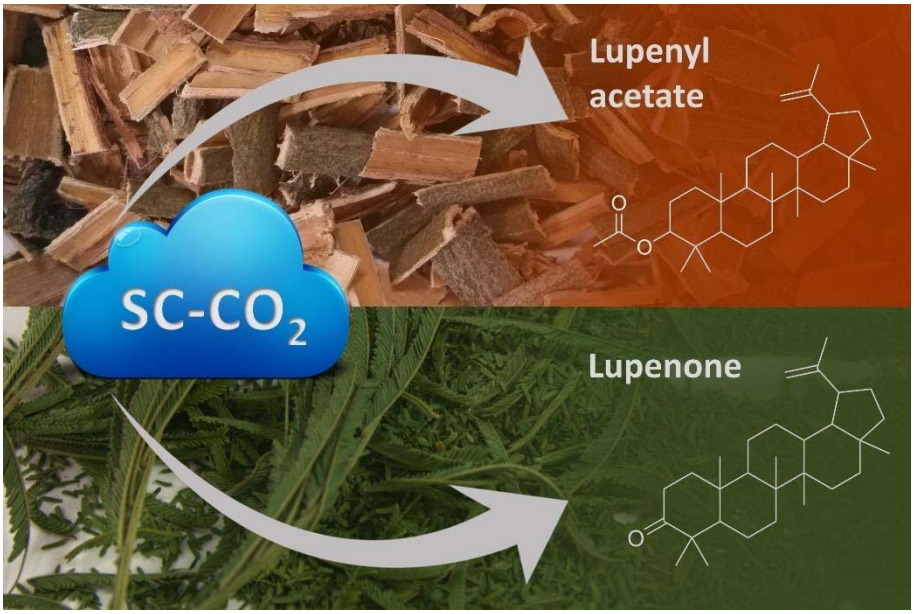
GRAPHICAL ABSTRACT



ABSTRACT

The species *Acacia dealbata* Link is a common sight throughout Iberian forests and its spread can be witnessed in most Portuguese provinces, occupying a total of area of *ca.* 8000 hectares [1]. For the existing flora this invasive species represents a threat as it can prevent their development, requiring the removal of its trees. While the resulting wood can be useful for many applications, the leaves, branches, and bark are commonly burn for energy production. In recent works [2,3], several triterpenoids of the lupane family were identified in the bark and leaves of this species, such as lupenone, lupenyl acetate and β-amyrone, which have been associated with valuable bioactive properties, namely, anti-inflammatory, anti-virus, anti-diabetes, among others [4]. The presence of these added-value compounds in what is considered a residual stream led to the study of a prospective source of extracts enriched with lupane-type triterpenoids, following the biorefinery and circular economy concepts using supercritical fluid extraction (SFE). The use of a green extraction method, such as supercritical fluid extraction combined with a green solvent, carbon dioxide, makes it compatible with the principles of green chemistry, prioritizing the use of benign solvents and auxiliaries [5].

The bark and leaves were extracted with supercritical CO_2, and the effects of pressure (100 – 300 bar), temperature (40 – 80 °C), and cosolvent addition (0 – 10 wt.%) using ethanol and ethyl acetate, were studied All extraction were carried out for the same time, flow rate of CO_2 and particle size. These assays were compared with conventional extracts obtained by Soxhlet using dichloromethane. The supercritical extractions of bark produced lower yields than those of leaves, which indicates a lower content of extractives, and is confirmed in the Soxhlet assays, which scored 1.4 and 3.8 wt.% for the bark and leaves, respectively. Nevertheless, the bark supercritical extracts were able to match the respective Soxhlet ones, which was not the case for leaves. The main triterpenoids observed in the bark were lupenyl acetate and lupenone, while the leaves presented a wider range of triterpenoids with lupenone being the main one, followed by β-amyrone and α-amyrone. In the case of bark, the SFE yields of lupenyl acetate and lupenone ranged from 10 to 800 mg kg_{bark}^{-1}. SFE yields of triterpenoids from leaves were one order of magnitude higher, ranging from 500 to 4800 mg kg_{bark}^{-1}. Triterpenoid's concentrations did not change as greatly as the yield. The increase of pressure was favorable for the total extraction yield in most cases, whereas the individual yields optimum conditions were found at the balance between pressure and temperature so that the solutes solubility could be improved without compromising the solvent power of the supercritical fluid. For both biomasses, the addition of ethanol as modifier only favored the total extraction yield, as the desired compounds did not improve their yield or concentration in the extract. The use of ethyl acetate as modifier favored both total and individual extraction yields, which was confirmed with higher concentrations of triterpenoids in the extracts. However, it only matched the best results obtained with pure CO_2 and did not show significant improvements for their individual yields and concentrations in the extract. Overall, this work provides an important step for the valorization of *A. dealbata* biomass following the biorefinery concept and highlights the importance of the experimental optimization for supercritical fluid extraction processes.

ACKNOWLEDGEMENTS

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Polymorphic control of indomethacin with the gas antisolvent method

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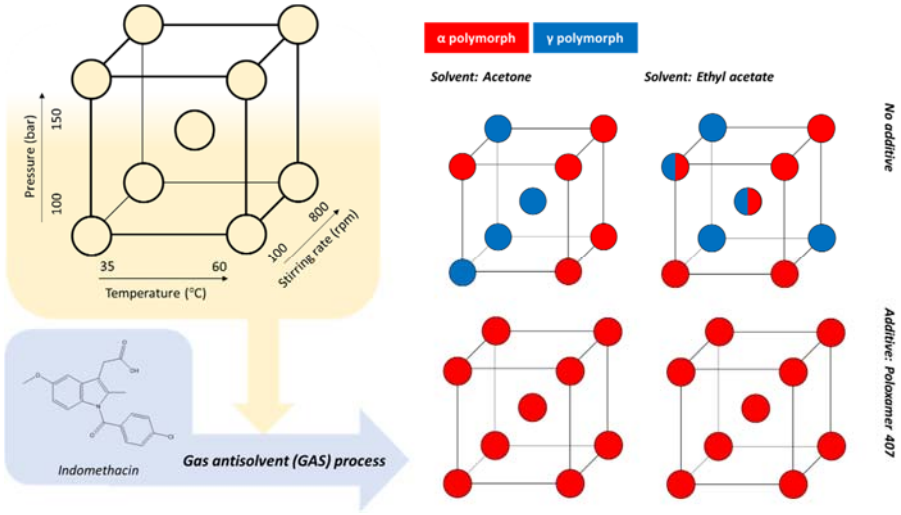
GRAPHICAL ABSTRACT



ABSTRACT

The polymorphic control of active pharmaceutical ingredients (APIs) is a major challenge in the manufacture of medicines for the pharmaceutical industry [1,2]. Crystallisation methods based on supercritical carbon dioxide as an antisolvent provide the ability to create unique solid forms of APIs, particularly with a tendency to generate metastable polymorphic forms [3]. In this work, effect of processing conditions of the gas antisolvent (GAS) method, such as pressure, stirring rate, temperature, type of solvent used, and the presence of an additive, were studied on the polymorphism of indomethacin. These variables were reported to potentially affect the solid-state, particle size, and morphology of the particles produced [4–6]. It was observed that a higher process temperature favoured the formation of the α polymorph of indomethacin. However, absolute control over the production of the α metastable polymorph of indomethacin was achieved only when poloxamer 407 was used as the additive. A detailed molecular modelling (including DFT, SAPT, enthalpy calculations and, molecular dynamics) study gave insight into the role of poloxamer 407 in the formation of the α polymorph and it concluded that the formation of the α polymorph is favored when this polymer is used as additive. This work shows that the GAS method enables control of the polymorphic form of indomethacin

using poloxamer 407 as a molecular additive. This work contributes to the knowledge of the production of different polymorphic forms of indomethacin using techniques based on supercritical CO_2.

ACKNOWLEDGEMENTS

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Towards the fabrication of medicinal nanovesicular formulations under GMPs

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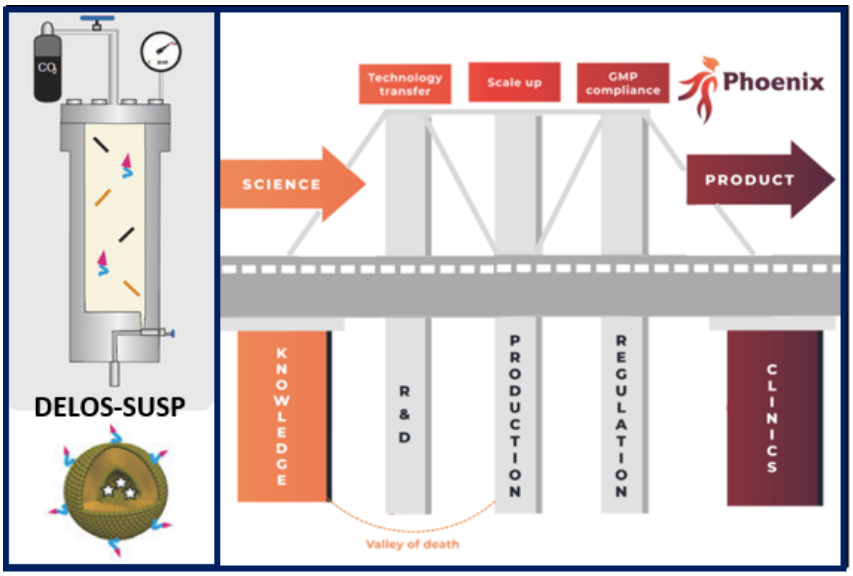
GRAPHICAL ABSTRACT



Adapted from [2]

ABSTRACT

The DELOS-susp method is a compressed fluid-based technology that allows the reproducible preparation of different nanovesicular systems with remarkable physicochemical characteristics in terms of homogeneity, morphology and particle size. DELOS-susp is a robust and green technology that uses compressed CO_2 as a processing fluid, which is inexpensive, highly available, non-toxic and non-flammable. In addition, CO_2 supercritical state is achieved at moderate temperature and pressure, which makes DELOS-susp a versatile technology to integrate different labile active compounds that can lead to innovative nanomedicines with enhanced efficiency [1]. All this advantages confer DELOS-susp-produced nano-pharmaceuticals, the potential to drive the scientific and technological uplift, offering great clinical and socio-economic benefits to the society in general, industry and key stakeholders and patients.

Nevertheless, the establishment of current good manufacturing practice (cGMP) in nano-pharmaceutical production at large scale, such as the ones produced with the DELOS-susp process, is a key step to successfully transfer nano-pharmaceuticals from lab to

industrial scale. Due to the lack of resources to implement GMP manufacturing at-site, the upscaling and production of innovative nano-pharmaceuticals is still challenging to main players of the EU nanomedicine market, start-ups and SMEs.

In this framework, we would like to present the PHOENIX-OITB [2], an European project that aims to establish a science- and regulatory-based Open Innovation Test Bed (OITB) in a way that would allow the timely and cost-friendly transfer of nano-pharmaceuticals from lab to industrial scale. PHOENIX-OITB will offer a consolidated network of facilities, technologies, services and expertise for all the technology transfer aspects from characterization, testing, verification up to scale up, GMP compliant manufacturing and regulatory guidance.

To test the operative capacity of PHOENIX-OITB, different demo-cases representative of different nano-pharmaceutical types, and different manufacturing methods and administration routes will be employed to demonstrate and verify the PHOENIX technologies in an industrially relevant environment. Two of these demo-cases are nanovesicular systems produced by the CO_2-based, DELOS-susp methodology. First, nanoliposomes loaded with an enzyme for intravenous administration have been developed for Fabry rare disease treatment. The entrapment of α-galactosidase (GLA) enzyme in these patented nanocarriers aims to improve the current enzymatic replacement therapy of Fabry disease, consisting in the intravenous administration of exogenous GLA to patients [3, 4]. Second, antimicrobial nanovesicles have been developed as potential nanomedicine for the topical treatment of microbial skin infections. This novel formulation, based on non-liposomal nanovesicles [5], exhibits inbuilt antimicrobial activity preventing infections, and has demonstrated to be effective against biofilms [6,7]. These two DELOS-based nanovesicular systems will be scaled up and manufactured under GMPs within the PHOENIX project to enable its clinical testing.

ACKNOWLEDGEMENTS

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Self-assembled silk fibroin aerogel particles for wound healing

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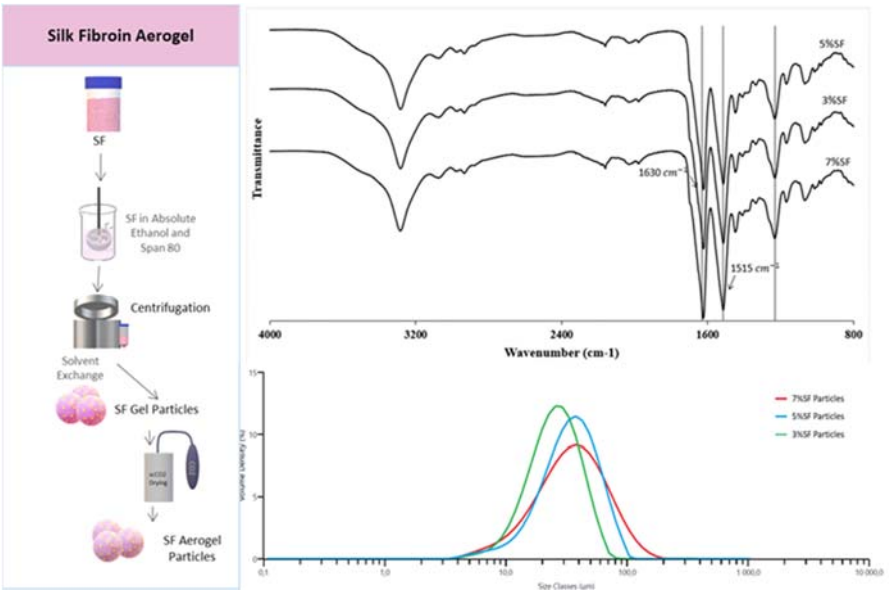
GRAPHICAL ABSTRACT



ABSTRACT

Chronic wounds are one of the major therapeutic and healthcare challenges. The design and development of biocompatible, biodegradable and adaptable materials that promote the tissue repair, prevent the infection and inflammation and ensure the control of exudate are a constant need for wound management [1]. Aerogels are nanostructured dry materials which can provide advanced performance for wound healing due to their high porosity, large surface area and water uptake, which can be tailored for a fast and directional fluid transfer of the exudate [2]. Aerogels can also act as carriers for bioactive compounds [1]. Silk fibroin (SF) is a protein that can be obtained from *Bombyx Mori* cocoons and has demonstrated to be an excellent stabilizer of bioactive compounds while supporting cell proliferation, being presently used in wound healing and regeneration [3]. In this work,

SF aerogel particles were developed and their potential as a controlled release system evaluated for wound healing applications.

SF extracted from *B. mori* cocoons was used to prepare SF aerogel particles. For the particles' production SF aqueous solutions at different concentrations (3, 5 and 7%(w/v)) were introduced into an absolute ethanol and Span 80 surfactant (3 wt.% with respect to SF) solution, followed by supercritical CO_2 drying. Ethanol was added at a ratio of 2:1 (v/v) in relation to SF solution. Span 80 was used as surfactant (3 wt.%) in relation to SF solution. The solution with Absolute Ethanol and Span80 was homogenized by mechanical stirring at 600 rpm and further SF solution added dropwise to the ethanol solution with stirring.

For the characterization of the SF particles, particle size distribution was determined by laser diffraction. Fourier Transform Infrared with Attenuated Total Reflectance (FTIR-ATR) spectroscopy was used to investigate the secondary structure formation, conformation and chemical structure. Textural properties have been performed by helium pycnometry and N_2 adsorption-desorption analysis.

The average diameter of the obtained particles varied at different concentrations. Particle diameter and dispersion increase with increasing SF concentration. According to the FTIR-ATR analysis, it was possible to verify the presence of the main characteristic bands of SF assigned to the presence of β-sheet structure, characterized by strong bands on the amide I and II regions. The physicochemical and textural characterization of the aerogels will allow to understand if this method is suitable to produce particles with adequate drug release kinetics. These particles constitute a drug delivery platform to be further loaded with pharmaceutical agents relevant for wound healing applications. *In vitro* tests are presently ongoing to validate the particles biocompatibility and suitability for wound healing.

ACKNOWLEDGEMENTS

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Supercritical carbon dioxide Salvia miltiorrhiza extraction: process optimization and analysis of bioactive compounds

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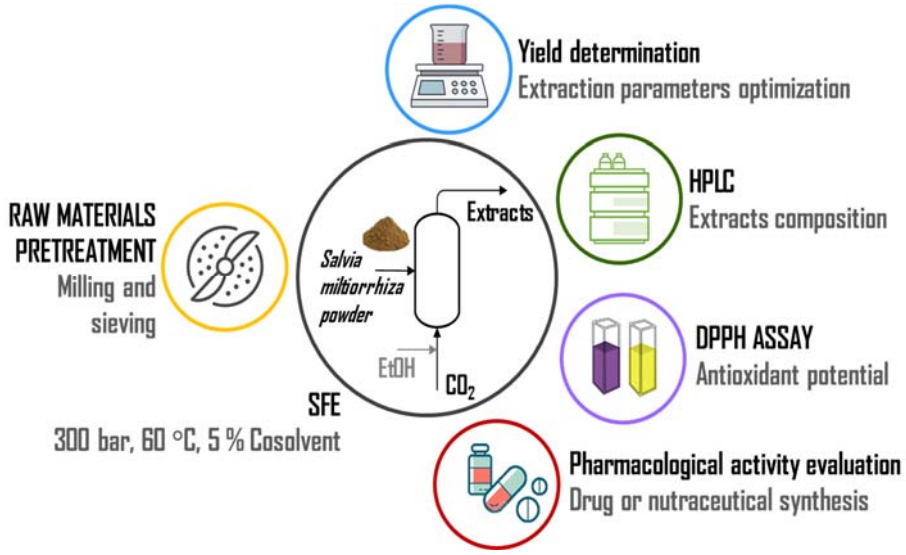
GRAPHICAL ABSTRACT



ABSTRACT

Salvia miltiorrhiza is a well-known traditional Chinese plant with plenty of medical values. In particular, the extracts obtained from the root of the plant present pharmacological properties such as antioxidant, antimicrobial, anticancer and anti-inflammatory activity. Its major bioactive lipophilic constituents are tanshinone I (TS-I), tanshinone IIA (TS-IIA) and cryptotanshinone (CTS), and the main bioactive hydrophilic constituent is salvianolic acid B (SAB) [1].

Supercritical CO_2 extraction has proven to be a very efficient technique for the extraction of bioactive substances derived from herbs and aromatic plants. Some works has proposed the feasibility of supercritical extraction of Salvia miltiorrhiza. In addition, due to the fat-soluble nature of tanshinones, extraction with CO_2 would be very appropriate for extracting them [2].

In this work, supercritical extraction of salvia has been developed at 300 bar, 60 ºC and with the use of 5 % EtOH as co-solvent [3]. To test the influence of particle size on the extraction yield, a grinding and sieving process of the raw material was carried out and the extracts obtained were analyzed by high performance liquid chromatography (HPLC)

to determine the amount of pharmacologically interesting compounds. In addition, the antioxidant capacity of the extracts was also analyzed using the DPPH assay.

The results obtained indicate that the two main compounds in the supercritical extracts are tanshinone IIA and salvianolic acid B. In terms of particle size, it was observed that the smallest size of raw material used in the extraction (4 mm) had the highest yield and the concentration in the majority compounds, tanshinone IIA, salvianolic Acid B, cryptotanshinone and tanshinone I, was higher than in cases where the root had been sieved to a larger size. Preliminary results of the DPPH analysis indicate that the Salvia miltiorrhiza extracts obtained by supercritical extraction have an inhibition percentage of about 60 %, which is a significant antioxidant capacity.

These introductory results indicate that Salvia miltiorrhiza could be considered a rich source of bioactive compounds, and once the extraction process has been optimized, the pharmacological activity of the supercritical extracts will be studied with the aim of synthesizing drugs or nutraceuticals.

ACKNOWLEDGEMENTS

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Poster Sessions

Extraction and Fractionation/ Purification

Pressurized liquids extraction for obtaining microalgae extracts enriched in carotenoids with anti-inflammatory and neuroprotective effects

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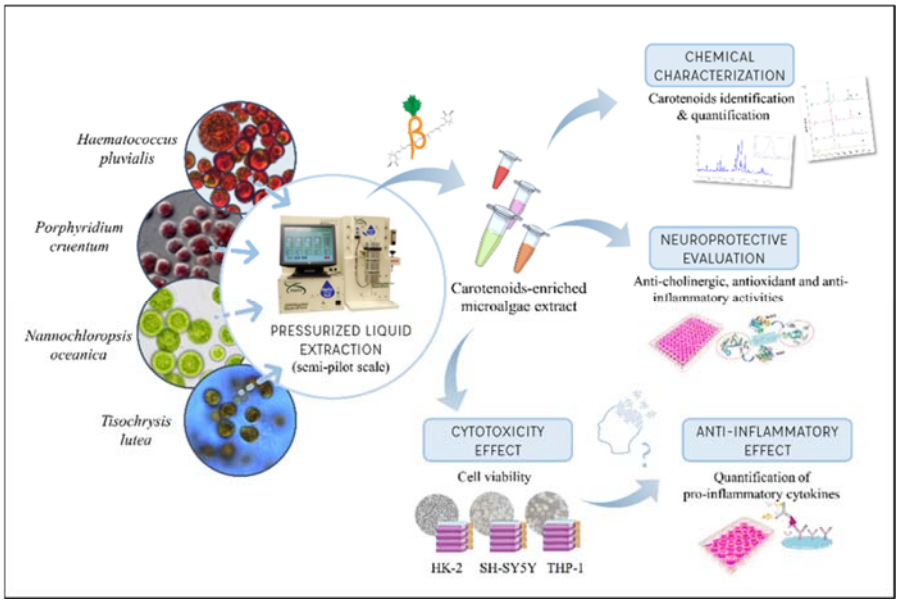
GRAPHICAL ABSTRACT



ABSTRACT

Microalgae are considered as an untapped reservoir for potential functional ingredients and high added-value compounds with diverse application in cosmetics, pharmaceutical and food industries [1]. Some of these bioactive compounds include polyunsaturated fatty acids, peptides, polyphenols, phytosterols or carotenoids [2]. Regarding carotenoids, these lipophilic compounds have been associated with an extensive list of health-promoting effects, including anti-inflammatory, neuroprotective or antioxidant properties [3], which emphasizes their potential value. In this study, the recovery of carotenoids from diverse microalgae, including extracts from *Haematococcus pluvialis*, *Nannochloropsis oceanica*, *Tisochrysis lutea* and *Porphyridium cruentum*, was performed using pressurized liquid extraction at semi-pilot scale, using the same optimum extraction conditions as obtained at lab-scale [4-7]. The extracts were chemically characterized by reversed-phase high-performance liquid chromatography with diode array detection (RP-HPLC-DAD) and then, these were evaluated through a battery of *in vitro* neuroprotective assays in an effort to estimate their potential against neurological disorders. Results indicated that microalgae extracts (obtained at semi-pilot scale) had

similar carotenoid profiles compared to extracts obtained at lab-scale, although higher yields were achieved due to the additional extraction cycle. All microalgae exerted a moderate and selective cholinesterase inhibitory potential, as well as high antioxidant and anti-inflammatory capacities, highlighting *N. oceanica* and *T. lutea* extracts. In parallel, cytotoxicity tests of the microalgae extracts were performed in different cell culture models, together with an *in vitro* evaluation of their anti-inflammatory capacity in THP-1 cells. In this regard, *N. oceanica* extract showed the highest inhibition of pro-inflammatory cytokine release, indicating that this microalga extract could be the most promising neuroprotective agent.

ACKNOWLEDGEMENTS

This work was supported by projects ABACUS (Algae for a Biomass Applied to the produCtion of added value compounds—funded by the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No 745668) and AGL2017-89417-R (MINECO, Spain). The authors thank Microphyt (Baillargues, France) and Algae for Future (A4F, Lisbon, Portugal) for kindly providing microalgae biomasses. A.V. would like to acknowledge the Spanish Ministry of Science, Innovation and Universities for his "Juan de la Cierva" post-doctoral grant (IJC2018-037560-I). J.D.S-M. would like acknowledge the Ministry of Education for a FPU predoctoral grant FPU17/01876. Z.J.S-M. would like acknowledge the University of Nariño (Colombia) for finantial support.

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Influence of pretreatments on the sub and supercritical-CO_2 extraction of usnic acid from *Usnea barbata*

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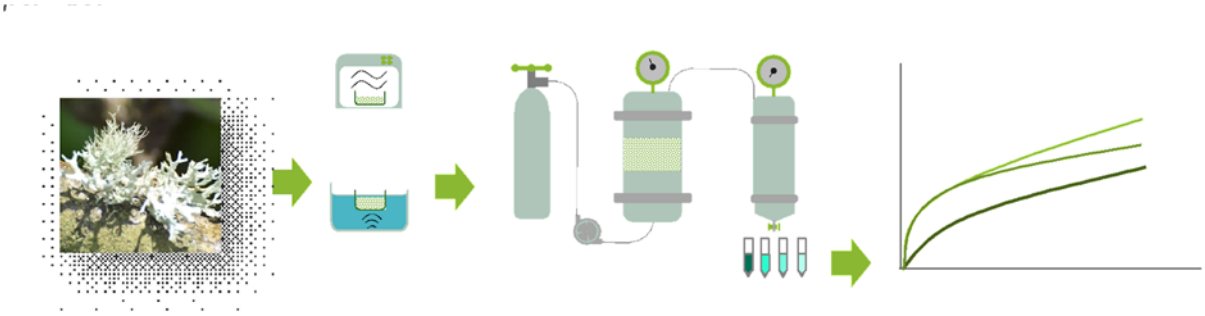
GRAPHICAL ABSTRACT



ABSTRACT

The mechanical pretreatment is a key factor on the extraction efficiency from plant, algal and mushroom materials. The unique structure of lichens and the irregular distribution of metabolites could led to an additional chemical variability [1, 2]. In lichens some metabolites are synthesized by the fungal partner and are deposited onto the surface [3], whereas others could be a part of the fungal cell wall or reserve compounds an found as intra and intercellular material. Due to these morphological particularities and the higher importance of the internal mass transfer compared to the external one, the disruption stage needs careful selection and a variety of particle size reduction techniques have been tried [3]. Furthermore, excessive material comminution should be avoided for industrial scale operation due to both the high energy requirements and plugging risks [3].

In this study, alternative conditioning techniques have been evaluated and compared to mechanical pretreatment for the extraction of usnic acid, a representative metabolite from a common lichen.

Samples from *Evernia prunastri* were collected in January 2021 in Ourense (Spain), air dried and ground in a knife miller at lab scale. Further powdering was performed on part of the sample, whereas other fraction was pretreated with microwave heating and another one was pretreated with ultrasounds. The different samples were then extracted under conditions, which were selected based in those reported previously to obtain high yields or purity, using liquid carbon dioxide 35 MPa and 40 °C and 5 % ethanol [4]. Kinetic studies allowed a comparison of the influence of the treatments on the different extraction stages, particularly on the internal mass transfer.

ACKNOWLEDGEMENTS

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Supercritical CO_2 extraction of tucumã-of-Pará oil rich in β-carotene on large scale: Experimental and mathematical modeling

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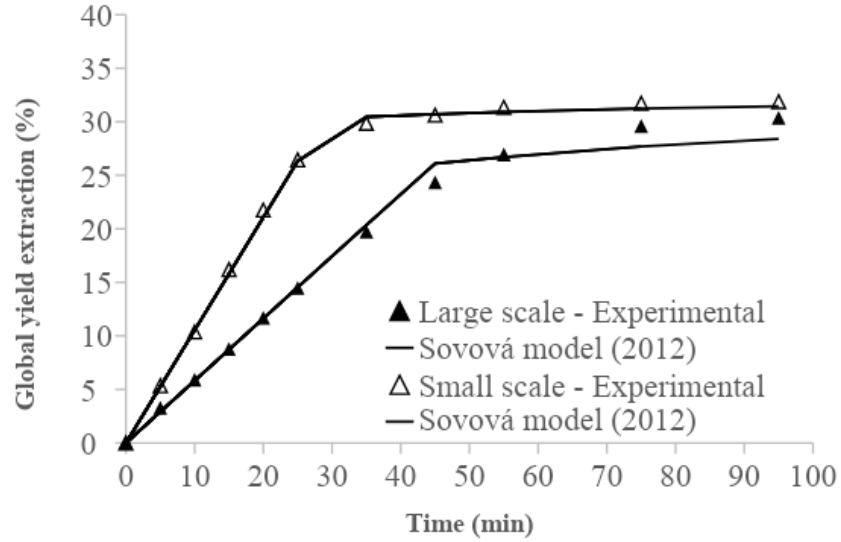
GRAPHICAL ABSTRACT



ABSTRACT

Supercritical fluid extraction is innovative, environmentally friendly, efficient, and has unique features that are extremely useful in scale-up development [1]. However, this scenario will always be challenging for scientists and engineers who seek to meet the demands of industrial production through the application of sustainable and economically viable technology for large-scale production and all these factors have motivated more specific studies that aim to understand the scaling up procedure by the interaction of solute and supercritical solvent from mathematical modeling [1, 2]. Thus, the aims of this work were to obtain Amazonian tucumã (*A. vulgare*), oil rich in β-carotene using supercritical CO_2 and shows how the kinetic parameters of the extraction, as well as the geometric dimension of the vessel of two extraction units, can influence the extract mass.

The experimental assays were divided in two steps: Experimental analysis and kinetic study. The operational conditions were 60°C combined with the pressures of 150 bar, 250 bar, and 400 bar, according to the same criteria described by Menezes et al. [3] and

Carvalho Jr. et al. [4] whose methodology kept the following variables constant: bed porosity (ε=0.59), apparent density (ρa=295 kg/m^3), and the ratio between bed height and bed diameter (H/D=3.66), from a small to a larger scale. The mathematical modelling was made by Sovová model [5] was used to determine kinetic parameters and mass transfer parameters.

On the small scale the highest extraction yield (31.9±0.2%) was obtained at 400bar with high concentration of oleic acid (approximately 65%) and with high β-carotene concentration (12220±24μg/g). On the scale-up, it was observed that the increase in solvent flow provided the obtainment of a smaller extract mass in the large scale compared to the small scale, during the first moments of extraction and this behavior may have caused a reduction in the contact between the solvent and the solute. These results are reinforced by the mathematical modelling, whose kinetic parameters show that the extraction time increased from 27 to 40 minutes from small to large scale. Furthermore, the fluid phase mass transfer coefficient was higher on the small-scale extraction ($6.5x10^{-5}min^{-1}$) compared to the large-scale extraction ($4.3x10^{-5}min^{-1}$) and these results justifies the increase in extraction time due to the reduction of the extraction rate from small to large scale. On the large scale the tucumã oil was obtained at 400bar with high extraction yield (28.4±0.2%), with high concentration of oleic acid (approximately 65%) and β-carotene concentration (15876±51μg/g). It can be clearly seen that increase in extraction time significantly enhances the β-carotene yields since it leads to enhance the time of the solvent with the solutes thereby enhancing the penetration and subsequent extraction of β-carotene from the sample matrix. Thus, the extraction process obtained satisfactory results.

ACKNOWLEDGEMENTS

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Supercritical CO_2 defatting of *Hermetia illucens* larvae

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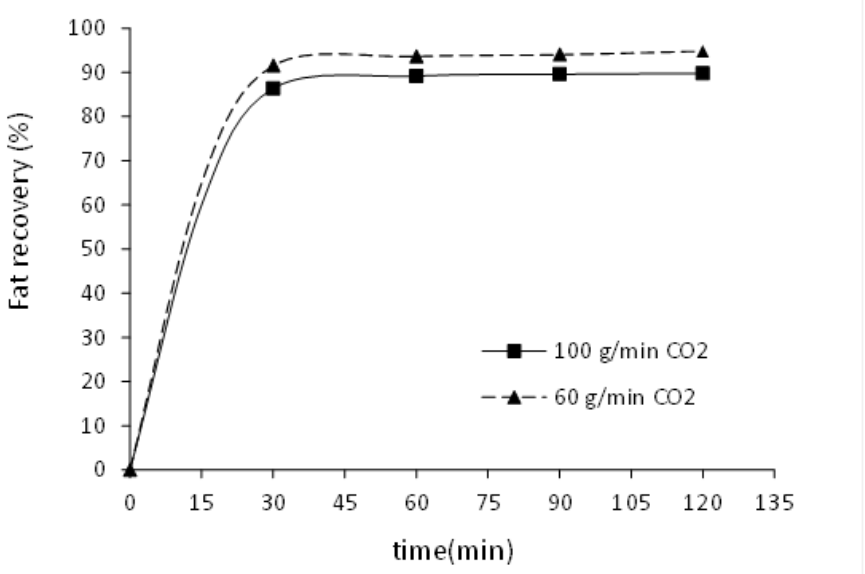
GRAPHICAL ABSTRACT



ABSTRACT

Edible insects are currently the most attractive novel source of proteins [1] with increasing applications in the UE for animal feeding and with recent approvals for human use of different insect species [2]. Furthermore, several recent works show evidences that edible insects are also rich in a great variety of minor bioactive compounds, with anti-inflammatory, antimicrobial, antiangiogenic, antiproliferative and/or antioxidant activity [3, 4]. Thus, edible insects may contribute significantly to human diet, with the advantage of being a sustainable natural source of food with a low environmental impact [5].

Several commercialized insect species contain high amounts of fat and thus, defeating becomes a required pre-treatment step in the manufacture of low-fat products and/or in the production of certain bioactive ingredients from insect larvae [6].

Black soldier fly (*Hermetia illucens*) dry larvae were milled and defatted using supercritical CO_2 extraction as an alternative to conventional defatting methods. The kinetic behavior at different extraction conditions was investigated in a 270 cm^3 extraction vessel containing 100 g of milled larvae with 47.3% fat (hexane extraction). At the

optimal conditions (60°C and 450 bar) the total fat removed from this sample in 30 min was 86.4% and 91.5%, respectively, using 60 and 100 g/min CO_2.

These optimal conditions were validated using another *Hermetia illucens* sample (different supplier), containing 34.5% fat (hexane extraction), in which case a 98.3% defatting was attained in 30 min with 100 g/min CO_2. Furthermore, the satisfactory results obtained in the low-scale defatting of *Hermetia illucens* larvae motivated the study of process scaling to an extraction vessel of 1350 cm^3 capacity (5-fold increase capacity) with c.a. 500 g of larvae, applying the semi-empirical approach presented by López Padilla *et al*. [7] in the supercritical extraction of a Colombian blueberry.

ACKNOWLEDGEMENTS

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Optimization of supercritical CO_2 extraction of phenolic and lipidic fractions from *Gigartina pistillata*

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GRAPHICAL ABSTRACT

ABSTRACT

Gigartina pistillata (S.G. Gmelin) Stackhouse is an underutilized carragenophyte with commercial potential for the production of hybrid carrageenans. In order to propose an integral utilization of resources, the valorization of other fractions of the seaweeds is encouraged [1, 2]. Despite seaweeds show low lipidic content compared to other terrestrial and marine sources, they are rich in long chain polyunsaturated fatty acids, increasingly demanded for their interesting nutritional and biological properties. Furthermore, red seaweeds show a higher ω-3 fatty acids content and lower ω-6/ω-3 ratio than other seaweed classes [3].

In order to extract biocompounds, such as phenolic compounds, supercritical CO_2 extraction using ethanol as modifier was proposed. Thus, the operational conditions during supercritical carbon dioxide extraction of commercially valuable fractions from the red seaweed *Gigartina pistillata* have been optimized.

This green technology offers advantages over conventional solvents, mainly derived from the shorter extraction times than conventional solvents and the possibility of obtaining a solvent-free lipidic phase also containing other bioactives. The selection of the operational conditions was addressed with response surface methodology according to a Box Behnken experimental design. The pressure, temperature and the ethanol concentration were optimized to maximize both the extraction yields and composition of the extracts [4].

ACKNOWLEDGEMENTS

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SPAGYRIA: Sustainable and Inclusive Production of Organic Skincare

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GRAPHICAL ABSTRACT

ABSTRACT

SPAGYRIA (EU Project € 1.8M) is an Interreg Poctefa Project dedicated to 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.

Agronomic trials were carried out in parallel in three different special employment centers –SECs– (Valentia-Huesca, Elkarkide-Pamplona and Les Jardins du Girou-Toulouse) to determine the best growing conditions of eight aromatic and medicinal plants. These preliminary agronomic parameters together with the first studies of supercritical extraction and concentration of active principles using carbon dioxide, made it possible to select the species for the preparation of the final cosmetic formulation.

Studies of extraction and concentration of active ingredients (Figure 1) of 5 different plant species were carried out using supercritical technologies. Supercritical extraction and anti-solvent fractionation with CO_2 are now presented in this work: *Salvia officinalis*, *Salvia sclarea*, *Calendula officinalis*, *Echinacea purpurea*, *Melissa officinalis*.

Figure 1. *Salvia sclarea* supercritical extract.

The results of supercritical extraction and concentration of actives using carbon dioxide as an anti-solvent agent indicate that clary sage, calendula and lemon balm as one of the most suitable for the preparation of ECO-cosmetic ingredients [1-3].

In this international meeting it will be presented for the first time, outside the scope of the project consortium, the final formulation authorized by the French drug agency –ASNM–. In order to achieve the commercial prototype, it was necessary to implement in our laboratory a complete traceability system for the samples and the production of technical and safety sheets for the extracts obtained.

During the last three months, clinical trials have been carried out with 60 volunteers at MEDES (French Institute for Space Medicine and Physiology). The results are currently being analyzed and the statistical conclusions will be released in the first quarter of next year.

We would like to emphasize that SPAGYRIA 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.

ACKNOWLEDGEMENTS

Authors thank EFA188/16/SPAGYRIA (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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Supercritical Antisolvent Fractionation of *Melissa officinalis* Extracts

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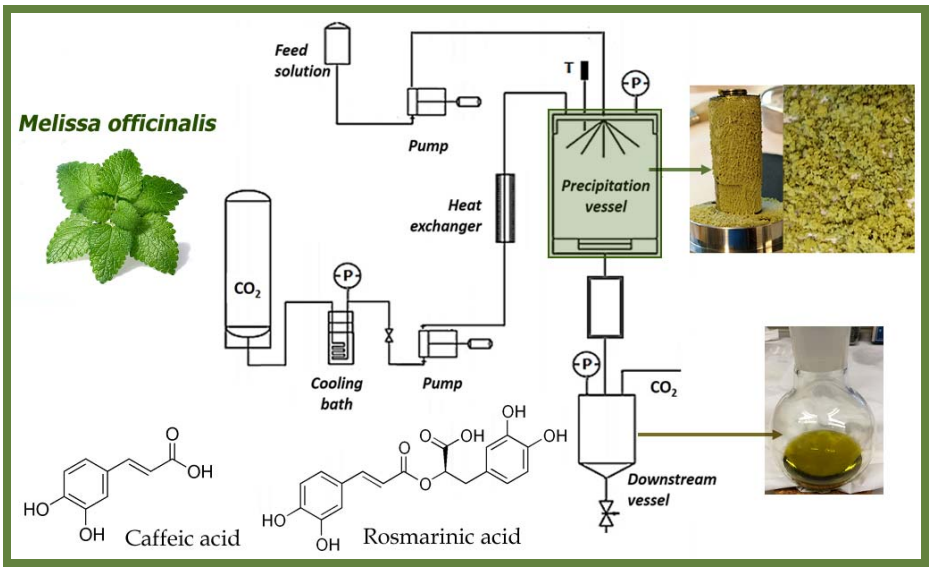
GRAPHICAL ABSTRACT



ABSTRACT

In recent years chemistry industry has focused on developing alternative processes with a less significant environmental impact. Traditionally, the extraction and separation of bioactive compounds has been carried out using conventional methods based on organic solvents but, as it 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. This supercritical fluid technology - and especially the use of carbon dioxide - has gained importance, being one of the most widely used methods for extracting, fractionating and isolating bioactive compounds from plants [1] or other natural sources as animal waste [2] due to its effectiveness, speed, use of moderate temperatures and environmental sustainability. In fact, due to these 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 [3].

This work focus on *Melissa officinalis*, a well-known Mediterranean species of the *Lamiaceae* family, which presents a wide and studied range of pharmacological activities [4,5]. Many of these properties have been attributed to bioactive compounds such as monoterpenes, diterpenes and phenolic compounds located in the leaves of this plant [6,7]. The objective of this study is to obtain and concentrate these bioactive compounds,

specifically antioxidants, from an organic cultivation of *Melissa officinalis*, which for the first time is going to be tackled by means of a sustainable double extraction with supercritical CO_2.

In this work, two supercritical techniques have been used to obtain, through a sustainable process, extracts of *Melissa officinalis* rich in phenolic compounds. For this, an extraction with supercritical CO_2 was first carried out and then a design of experiments was carried out to optimize and to study the variables of pressure and CO_2 flow. The results obtained show that it is possible to successfully obtain extracts rich in rosmarinic acid and caffeic acid from *Melissa officinalis*. In other words, a fine powder highly enriched in antioxidants and free of organic solvents is obtained in the chamber fraction, with potential applications in industries such as cosmetics, food or pharmaceuticals.

ACKNOWLEDGEMENTS

Authors thank EFA188/16/SPAGYRIA (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) and Gobierno de Aragón: Departamento de Ciencia, Universidad y Sociedad del Conocimiento (Group E39_20R)**.**

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Supercritical CO_2 Extraction and Purification of Bioactive Compounds in Olive Leaf with Molecularly Imprinted Polymers

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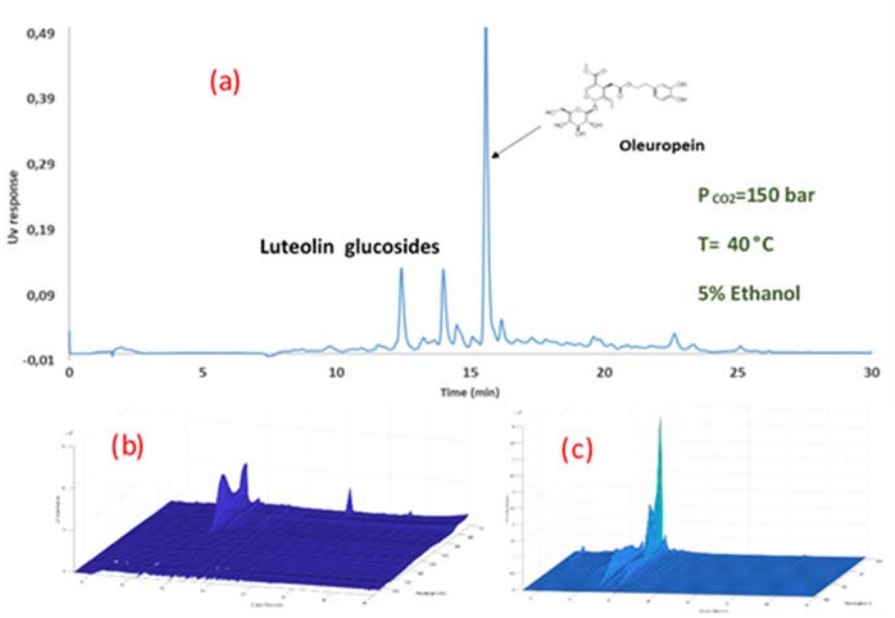
GRAPHICAL ABSTRACT



ABSTRACT

Around 4.5 million tons of leaves are generated each year as a by-product of the olive and olive-oil production. These olive leaves must be removed from the fields and olive-mills. Usually they are burned, or else used as fuels or for animal feed. However, olive leaf is extremely rich in bio-resources, being estimated that around 1 million tons of bioactive compounds, 1 million tons of cellulose and 1.5 million tons of lignin are currently underexploited [1]. In particular, the extraction and isolation of high-added value bioactive compounds in olive leaf (e.g. polyphenols, triterpenoids, essential oils, lipids, lignocellulose) is especially appealing due to their high-market potential in the food, feed, chemical, nutraceutical, cosmetic and pharmaceutical sectors [1].

This research combines the extraction of bioactive compounds in olive leaf using supercritical CO_2 with the subsequent processing of the extracts by sorption/desorption in

Molecularly Imprinted Polymers (MIPs), aiming at the isolation and purification of target compounds. Supercritical CO_2 extractions were performed in a range of operating conditions with temperature between 35 and 50 °C and pressure changing from 100 to 170 bar. Additionally, the presence of a co-solvent (e.g. ethanol, ethyl acetate), in a range of 0 to 10% (wt% with respect to CO_2), is also considered for assessment of their effect on the profile of the extracted compounds. HPLC-DAD and LC-MS are used for the identification/quantification of the bioactive compounds extracted with the different supercritical operation conditions, as illustrated in the graphical abstract for a run with P_{CO2}=150 bar, T=40 °C, 5% ethanol. In this extract, oleuropein and luteolin/luteolin-glucosides are the majority compounds but oleoside, verbascoside and oleanolic acid, among others, were also identified in the different extracts produced.

Due to their improved specificity, the efficient isolation/purification of bioactive compounds in plant extracts, namely in olive leaf extracts, is being nowadays considered with MIP adsorbents [2-4]. In this research we are designing and synthesizing MIPs to target compounds in olive leaf extracts considering the combination of different functional monomers (e.g. 4-vinylpyridine, methacrylic acid, acrylamide, styrene, etc) with capacity for H-bonding donor/acceptor interactions and π-π stacking with the template molecules (e.g. oleuropein, luteolin, oleanolic acid). Potential oxoanion interactions with urea groups are also being considered using a functional monomer synthesized from the condensation of 4-vinylaniline with 3,5-bis(trifluoromethyl)phenyl isocyanate. Morphology of the MIP particles is being tailored considering precipitation and suspension polymerization [3,4]. Furthermore, synthetic core-synthetic shell and cellulose core-synthetic shell [5] MIP particles are also being developed to enhance surface imprinting. Atom Transfer Radical Polymerization is being used for the generation of the hybrid cellulose-synthetic MIPs [5]. The different kinds of MIPs particles are assessed in sorption/desorption processes involving standard molecules and also real olive leaf extracts, namely with high pressure continuous processes. The results obtained show the feasibility of the proposed approach for the valorization of the olive leaf by-products as illustrated in graphical abstract b/c with the separation of luteolin and oleuropein enriched fractions.

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Bioprospecting neuroprotective extracts from Algerian plants using green compressed fluid extractions

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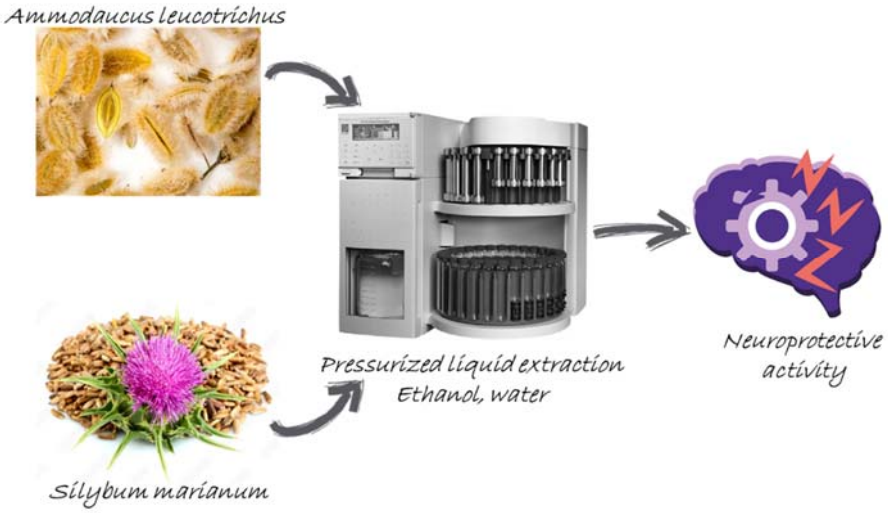
GRAPHICAL ABSTRACT



ABSTRACT

Bioprospecting can be defined as the organized and systematic exploration for products found in the natural environment that have some commercial scientific potential or medical value [1]. This is almost nonexistent in Africa comparing to the others continents. As case of study, we select Algeria, which is the largest country in Africa, it is characteristic by the diversity of climate, geology and soil that is reflected on the biodiversity of the region.

The objective of numerous investigation has been to improve novel and efficient extraction techniques to isolate high-quality molecules respecting the Green Chemistry Concepts. In this regard, extraction using compressed fluids such as pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) using water, ethanol and CO_2 as green solvents is being an excellent choose for the extraction of bioactive molecules from different matrices [2].

Neurodegenerative diseases, such as Alzheimer (AD), are defined by the progressive and slow dysfunction of neurons and axons in the brain [3]. The pathological mechanism

operating in AD have gained more attention, due to the huge number of population that is suffering, around 45 million worldwide. Neuroinflammation and cholinergic lack are key detrimental processes involved in AD [4]. Therefore, acetylcholinesterase (AChE) and lipoxygenase (Lox) inhibitors are an important strategy to prevent neurodegenerative disorders.

In the present work, during bioprospecting studies, *Ammodaucus leucotrichus* (AL) and *Silybum marianum* (SM) were treated with different Pressurized Liquid Extraction (PLE) conditions to obtain neuroprotective rich extracts. The main parameters influenced different extraction techniques have been optimized. Moreover, the extracts obtained have been chemically and functionally characterized and in-vitro assays such as the anti-inflammatory and anti-cholinergic activities. Results indicated that the best IC_{50} of AChE value obtained was 55.59 µg/mL using PLE with water from AL seeds at 180 °C, 10.34 MPa (1500 psi) using a extraction time of 10 min, while, the best inhibition of Lox was 32.42 µg/mL using PLE with 50% ethanol from SM seeds at 75 °C, 10.34 MPa and 10min. The findings reinforce the demand for further research on the other biological activities and deep chemical caracterization of bioactive molecules.

ACKNOWLEDGEMENTS

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Highlighting the bioactivity of kiwiberry leaves extracts as cosmetic ingredient – An exploratory *in vitro* study

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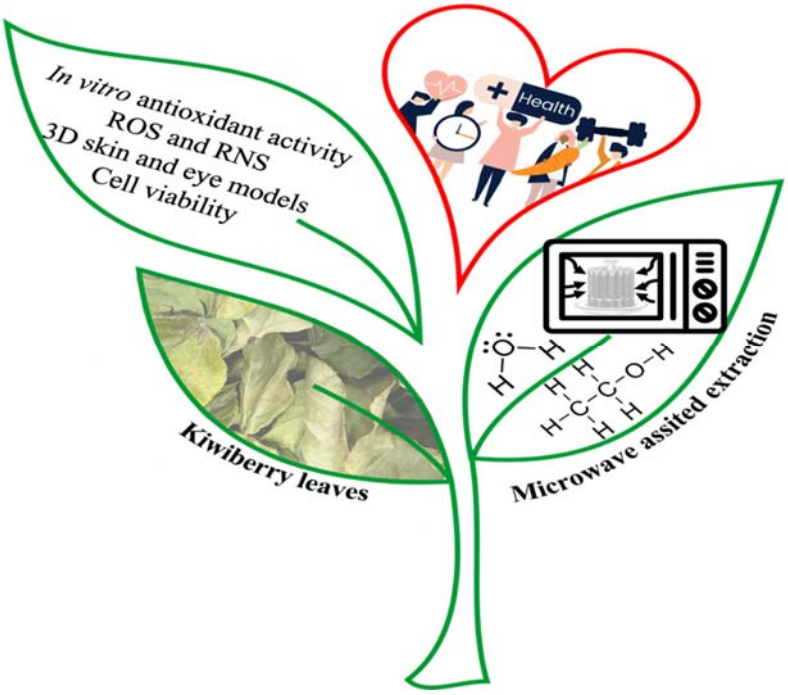
GRAPHICAL ABSTRACT



ABSTRACT

Kiwiberry (KB) is a small grape-sized fruit, characterized by hairless skin and a pleasant aroma and flavor. Worldwide, the production of KB is increasing mainly due to the biological properties (such as antioxidant activity) associated. The most recent data stated that in 2015/16 the worldwide production was almost 1600 tons [1]. In Portugal, the national production achieved a record value of 120 tons in 2016 [1]. Nevertheless, during KB production different by-products are generated, such as fruits without caliber to be commercialized, pomace, or leaves [2]. The aim of this work was to extract bioactive

compounds from KB leaves using a green and eco-friendly technique: Microwave-assisted extraction (MAE). Therefore, compatible food solvents were employed, namely water, ethanol, and a mixture of both (1:1). The alcoholic extract achieved the highest phenolic and flavonoid contents (629.48 mg of gallic acid equivalents (GAE) per gram of plant material on dry weight (dw) (GAE/g dw) and 136.81 mg of catechin equivalents per gram of plant material on dw (CAE/g dw), respectively). Oppositely, the hydroalcoholic extract achieved the highest antioxidant/antiradical activity (IC_{50} = 95.22 μg/mL and 131.58 μg/mL for DPPH and ABTS assays, respectively) as well as scavenging capacity against reactive oxygen and nitrogen species (IC_{50} = 29.10 μg/mL for $O_2^{\bullet-}$; IC_{50} = 1.87 μg/mL for HOCl and IC_{50} = 1.18 μg/mL for $^{\bullet}NO$) [3]. Considering these results and the bioactive composition, the hydroalcoholic extract was selected to be screened for a potential cosmetic application. A cell viability assay was performed in keratinocytes (HaCaT) and fibroblasts (HFF-1) (0.1 - 1000 μg/mL). The MTT results revealed that the hydroalcoholic extract did not decrease viability of HaCaT, while HFF-1 viability was above to 90%. The 3D skin and ocular models were applied to evaluate the irritant potential. The viability achieved in both models were, respectively, 55.18% and 101.15%. The IL-1α released for the skin and ocular models were, respectively, 0 pg/mL and 35.60 pg/mL, being the extract classified as non-irritant for both models. The elastase and hyaluronidase are enzymes involved in skin aging process; thus, inhibition capacity of the promising extract was evaluated. The inhibitory effects on elastase and hyaluronidase, enzymes involved in skin aging process, were also tested, leading to inhibition of 65.62% and 54.64%, respectively, for a concentration of 1 mg/ml.

These results highlight that KB leaves extracted with MAE are a promising cosmetic ingredient, being not irritant to skin application. Nevertheless, further studies must be performed to ensure the skin *in vivo* effects (such as anti-wrinkle) in consumers.

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Salicornia ramosissima: A possible green alternative with valuable bioactive properties for cosmetics

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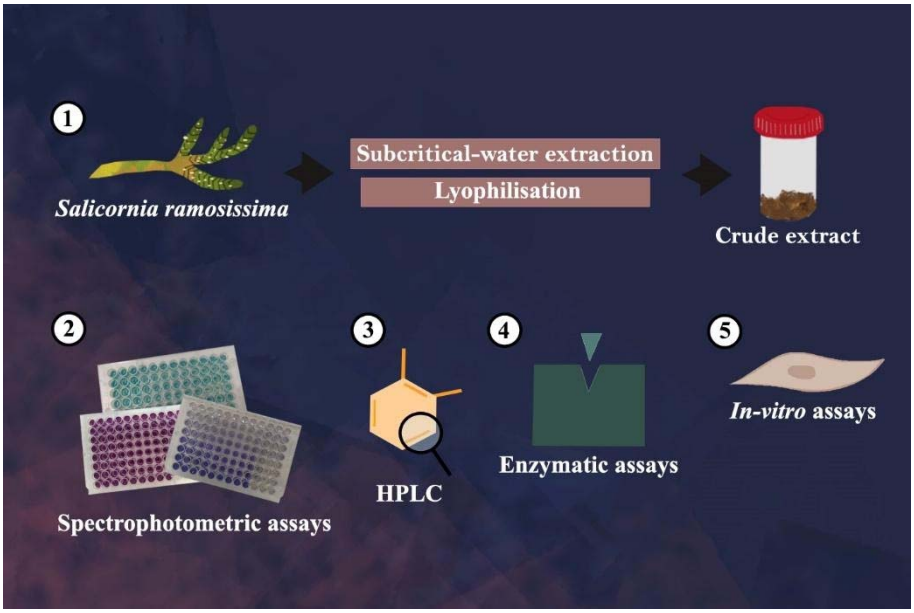
GRAPHICAL ABSTRACT



ABSTRACT

The cosmetic industry has been continuously growing worldwide, being one of the biggest and successful industry [1] with a worth of USD 277.67 billion in 2020, according to Fortune Business Insights. In recent times, consumers have been more aware of environment and sustainability issues, having deep concerns related with the products origin and production process as well as their ecological implications and safety [2,3]. Therefore, the search of natural products as cosmetic ingredients has been a focus for cosmetic industry [4]. Since consumers correlate botanicals with safeness, mainly due to its origin, these ingredients began to constitute desirable compounds over synthetic ones for cosmetic formulations [5,6]. *Salicornia ramosissima,* popularly known as green samphire, is a halophyte plant widely distributed [7], [8]. Notwithstanding being consumed [9], halophytes still remain under valorized regarding its bioactive composition, being rich in antioxidants [10] and lipid components, such as essential fatty acids [11]. This study aims to extract and validate a new active ingredient from *S. ramosissima*, through *in-vitro* and *ex-vivo* assays, in order to highlight its potential use in skin formulations. The halophyte's extracts were obtained by subcritical-water extraction

(SWE) at different temperatures (110, 120, 140, 160 and 180 ºC) and the antioxidant and radical scavenging activities as well as the phenolic profile were screened. The sample extracted at 180 ºC presented the highest phenolic content (1739.28 mg/100 g dw), along with phenolic acid predominance (1054.77 mg/100 g dw). Despite not being efficient in the sequestration of the radical $ABTS^{+\cdot}$, this sample was the only that reasonably sequester the $DPPH^{\cdot}$ (IC_{50} = 824.57 μg/mL). The scavenging capacity of superoxide ($O_2^{\cdot-}$) and hypochlorous acid (HOCl) were also considerable (respectively, IC_{50} = 158.87 µg/mL and IC_{50} = 5.80 µg/mL). The results obtained support the bioactivity of *S. ramosissima* and its possible use as a cosmetic ingredient. Further studies, particularly enzymatic activity assays and *in-vitro* assays on skin cell lines, should be performed to support this new application.

ACKNOWLEDGEMENTS

This work received financial support from project EXPL/BAA-GR/0663/2021 – Kiwi4Health – Exploring the Eco-Innovative Re-Use of Kiwiberry, supported by national funds by FCT/MCTES. The work was also supported by UIDB/50006/2020 and UIDP/50006/2020 by Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. Ana Margarida Silva is thankful for the PhD grant from Portuguese Foundation for Science and Technology (SFRH/BD/144994/2019). Manuela M. Moreira (project CEECIND/02702/2017) is grateful for the financial support financed by national funds through FCT and to REQUIMTE/LAQV. Francisca Rodrigues is thankful for her contract (CEECIND/01886/2020) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.

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Neuroprotective compounds recovered by pressurized liquid extraction from agrifood by-products.

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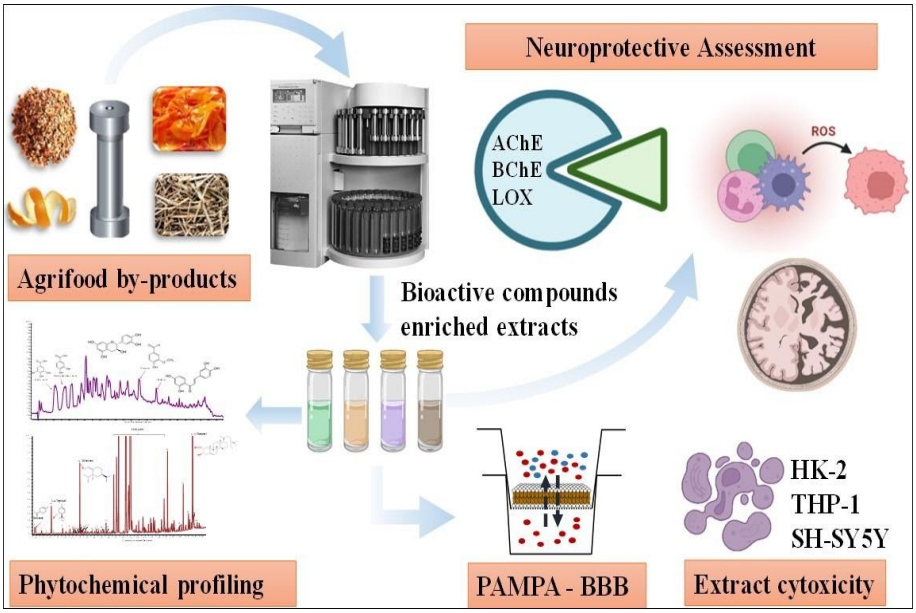
GRAPHICAL ABSTRACT



ABSTRACT

Agricultural and food industries generate several tons of wastes that represent a large burden for environment and economic governance. However, these residues can be considered as a renewable and low cost source of bioactive compounds[1]. In this sense, revalorization of agrifood by-products like seeds, leaves, peels, weeds and woods using green extraction processes such as pressurized liquid extraction (PLE) involve an ideal opportunity to recover bioactive molecules from bio-wasted materials[2]. Among these bioactive molecules, terpenoids and phenolic compounds have been described as potential neuroprotective molecules due to their anticholinesterase, antioxidant and anti-inflammatory capacities[3–6]. In the present study, several extracts from *Citrus sinensis, Robinia pseudoacacia, Nothofagus pumilio, Cyphomandra betacea* and coffee silverskin by-products obtained by PLE optimized conditions, were chemically characterized by the use of gas and liquid chromatography coupled to mass spectrometry (GC/LC - MS) and their *in-vitro* neuroprotective capacity was also evaluated using a set of bioactivity assays related to neurological disorders such as Alzheimer's Disease. In parallel, central nervous system accessibility was evaluated making use of an *in-vitro* model of parallel artificial membrane permeability assay for the blood–brain barrier (PAMPA-BBB). Moreover, *in-*

vitro cytotoxic effect of evaluated extracts was tested in different human cell culture models (HK-2, THP-1 and SH-SY5Y cells). Results showed promising *in-vitro* neuroprotective effect in all the extract tested, however small bioactive molecules such as phenolic acids and monoterpenoids showed more permeability in the BBB model employed. Moreover, the cytotoxic evaluation placed *Citrus sinensis*, *Cyphomandra betacea* and coffee silverskin extracts as non-toxic extracts.

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Phytochemistry, metabolomics, and biological activity of *Gypothamnium pinifolium* Phil. from Northern Chile

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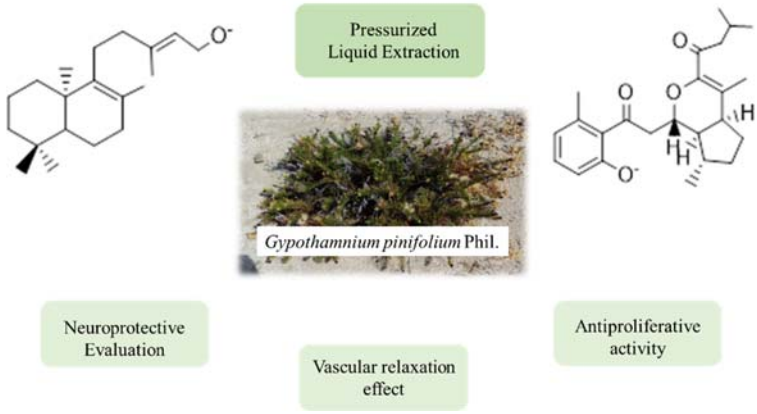
GRAPHICAL ABSTRACT



ABSTRACT

Noncommunicable - or chronic- diseases (NCDs) affect millions of people worldwide, constituting an important health problem. In the last years, the use of enriched or phenolic-concentrated extracts from native plants is of high for ameliorating or preventing NCDs. These pathologies are long duration diseases, generally with slow progression, being cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes the main types (1). These diseases, along with neurodegenerative diseases, are a public health problem accounting for nearly three quarters of deaths, which represents a 71% of the global disease burden worldwide in general, with economic lost around $47 trillion for the period 2010–2030 (2). Therefore, strategies for the prevention of these pathologies have become a priority during the last years, as stated in the strategy for the

prevention and control of NCDs by the World Health Organization (3). In this context, the endemics plants from Chile represent an underexplored source of biomolecules that can become potential candidates for the study of new active principles, supporting its use as functional food ingredients or nutraceuticals. *Gypothamnium pinifolium* Phil. (Asteraceae) is a Chilean native small shrub growing in the Paposo Valley, one green spot in the coast of The Atacama Desert; this species produce rare phenolic compounds including 2-nor-1,2-secolicoserone, one 5-methyl-coumarin: aphillodenticulide, and one labdane: ent-labda-8,13-E-dien-15-ol (4). The present study was performed using conventional extraction and pressurized liquid extraction (PLE), also, high-resolution phenolic fingerprinting and antioxidant, enzyme inhibitory potential and antiproliferative activities were studied. Moreover, the hypotensive effects in rat aorta from *G. pinifolium* extracts and its two major compounds were investigated for the first time. The phenolic compounds of this plant were isolated by chromatography to test in antioxidant and inhibitory enzymes assays. In addition, a complete metabolomic profile was performed by HR-MS. Using ultra-high performance liquid chromatography-photodiode array detection hyphenated with Orbitrap mass spectrometry analysis, several metabolites were identified including specific coumarins. Good inhibition against acetylcholinesterase, butyrylcholinesterase and tyrosinase enzymes was found for the hexane extract and main isolated compounds. In addition, antioxidant activity was assessed using bleaching of DPPH and ABTS and ORAC experiments for the extracts. The antiproliferative potential against several solid human cancer cells was also investigated for the extracts and the two main compounds, one coumarin 2-Nor-1,2-secolycoserone and one diterpene ent-labda-8,13-E-diene-15-ol, which showed to be active against some cancer lines. Our findings suggest that *G. pinifolium* is a rich source of bioactive coumarins with potentiality against chronic noncommunicable diseases.

ACKNOWLEDGEMENTS

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Extraction of phenolic compounds from Brazil nut (*Bertholletia excelsa* HBK) skin using pressurized liquid extraction

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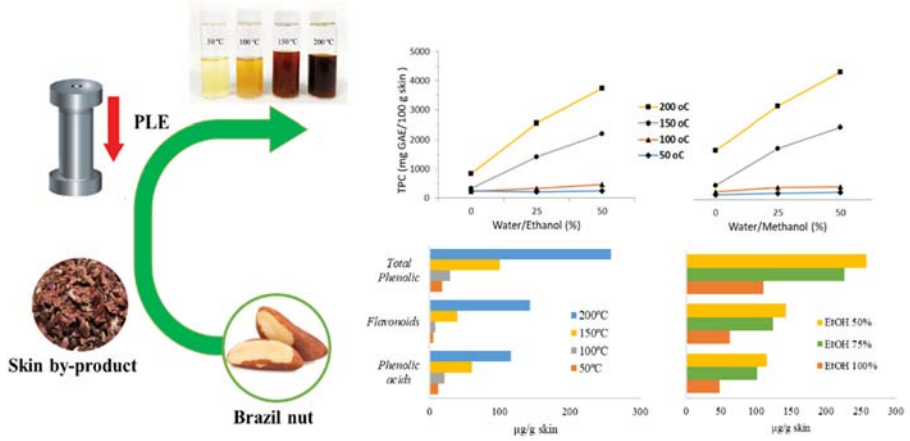
GRAPHICAL ABSTRACT



ABSTRACT

The skins of nuts represent an important source of fiber and phenolic compounds with diverse bioactive properties such as antioxidant, antimicrobial, anti-inflammatory and cytoprotective [1]. Brazil nut (*Bertholletia excelsa* HBK) is a nut native of the Amazon rainforest with large industrial production in Brazil, Bolivia and Peru, which in turn generates large amounts of by-products such as skin (BNS), with no outstanding value at present [2]. BNS is known as an important source of phenolic compounds and potential bioactive ingredient [3], but few extraction studies have been conducted. This work evaluates the pressurized liquid extraction (PLE) technique under different temperature conditions and solvents (water-alcohol mixtures), and the effect on total phenolic compounds (TPC) and type of phenolic compounds recovered.

The results show he important influence of temperature and the addition of water to alcohol (methanol and ethanol) on TPC content. At 50% (v/v) water/alcohol mixture, the highest TPC were obtained: at 200ºC, it was 3738 to 4348 mg GAE/100 g skin; and at 150ºC, it was 2206 to 2478 mg GAE/100 g skin. In addition, the temperature produced a change in the phenolic profile, as observed in water/ethanol 50%, phenolic acids are predominant up to 150ºC and at 200ºC were flavonoids. In addition, the water/ethanol mixture (50 and 25% v/v water) had a higher extraction efficiency of phenolic acids and flavonoids than the pure alcohol. The positive effect of water/alcohol mixtures and high temperatures on TPC was also reported in the extraction other plant by-products [4, 5],

and is attributed to the improvement of analyte solubility and solvent diffusivity, enhancing solvent penetration into the plant matrix and mass transfer.

Thus, PLE at temperatures of 150-200°C using water-alcohol mixtures is a suitable technique for the extraction of phenolic compounds from BNS, particularly flavonoids. Nevertheless, it is necessary to consider, the possible formation of Maillard reaction products due to the observed color change. The results obtained promote the use of Brazil nut agro-industrial by-products to the development of new ingredients in the food and/or pharmaceutical sectors.

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High-pressure assisted extraction of levodopa from Mucuna pruriens seeds

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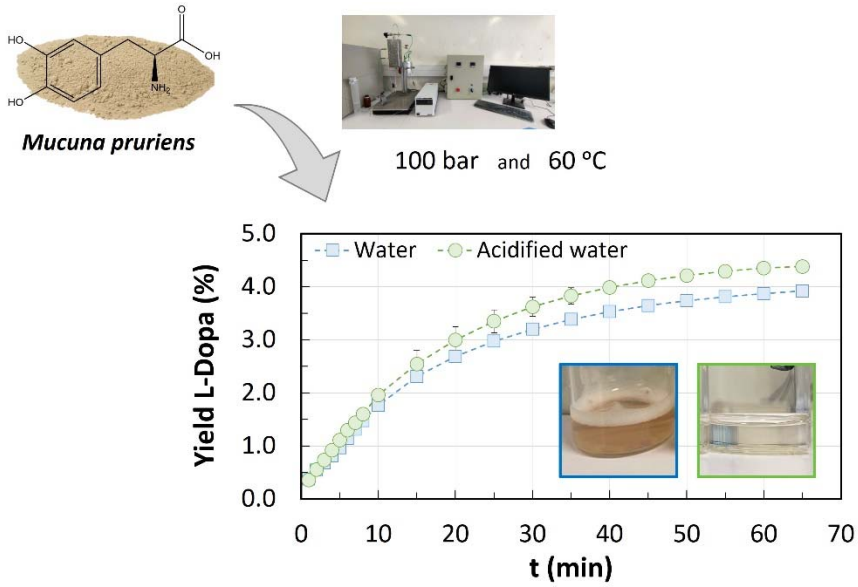
GRAPHICAL ABSTRACT



ABSTRACT

Levodopa is an amino acid commonly found in plants, such as *Mucuna pruriens*, and is used in the treatment of Parkinson's disease. Classically, the best attempts to extract levodopa from biomass been achieved using methanol, ethanol:water mixtures in presence of ascorbic acid, chloroform in alkaline media, and acetonitrile. Aiming to find more efficient extraction processes and identify more sustainable solvents, in this work, high-pressure-assisted extraction and aqueous solutions of carboxylic acids were combined to extract levodopa from *Mucuna pruriens* seeds. First, a screening of the most suitable acids and their concentrations was performed at 100 bar, 2 mL/min, 60 °C and 60 minutes, with 10% citric acid by weight leading to higher yields and purity of levodopa. After, the effect of the pressure in the yield and purity of levodopa in the extracts obtained was also studied (20 bar to 100 bar), applying the most promissory solvent. Finally, a screening using other extraction techniques, namely a conventional extraction and the alternative techniques of ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) was also performed, in order to understand the advantages of each.

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Mathematical modelling and simulation of reconditioning processes in supercritical CO_2 extraction

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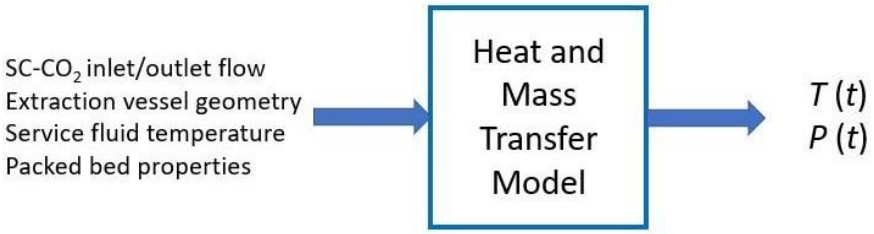
GRAPHICAL ABSTRACT



ABSTRACT

SuperCritical (SC) carbon dioxide (CO_2) extraction is a process carried out at high pressures, being this a disadvantage to operate continuously with solid substrates [1]. However, industrial SC-CO_2 extraction plants use two or more extractors to approach a continuous operation by placing one extractor in reconditioning process while the rest is in service [2]. The reconditioning process has four steps: depressurization, unloading of extracted substrate, loading of fresh substrate, and repressurization of the system. Because the allotted time (t_r) for this process is limited by the extraction time and the number of the extractors, it is important to estimate the optimum time to accomplish the objective of reconditioning the extractor while considering the constraints of this process (*e.g.*, avoid an abrupt drop in the temperature of the extractor wall due to a faster depressurization) and calculate the initial conditions for the subsequent extraction.

The objective of this work is to model and simulate the heat and mass transfer in the processes of depressurization and pressurization in the operation of a SC-CO_2 extraction plant, and to calculate the reconditioning time along with the pressure and temperature profiles along the whole process.

The heat and mass transfer models were obtained through a heat and mass balance within the extraction vessel, respectively. The change in the mass of SC-CO_2 within the extraction is given by the inlet or outlet flow (according to the case), and from this equation the fluid density can be estimated. The change in the temperature of SC-CO_2 is estimated with the heat flows by convection from wall-to-fluid and substrate-to-fluid transfer phenomena and the enthalpy of the inletting or outleting flow. The pressure of

the system is estimated as function of the fluid density and temperature through Equations of State using REFPROP [3].

The model allowed estimating the temperature and pressure profiles within the extraction vessel during the depressurization and pressurization processes as a function of the initial conditions of the system and the inlet or outlet flows. There was an abrupt decrease in temperature and pressure of the system at the beginning of the evacuation during depressurization due to the Joule-Thomson effect caused by the expansion of the SC-CO_2 at the outlet of the extractor, then the temperature increased due to the heat transferred from the wall and the substrate and the pressure decreased at a slower rate to avoid the formation of a liquid-gas mixture. The temperature also dropped at the beginning of pressurization due to the expansion of the SC-CO_2 stream into empty packed bed extraction vessel, and subsequently increased when the previously described heat flows overcame the expansion effect. During pressurization the pressure increased at a lower rate when the fluid was in a gaseous state and at a higher rate when the CO_2 reached the supercritical state.

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Conceptual design of a high-pressure packed column for the supercritical CO_2 fractionation of glycerol acetates

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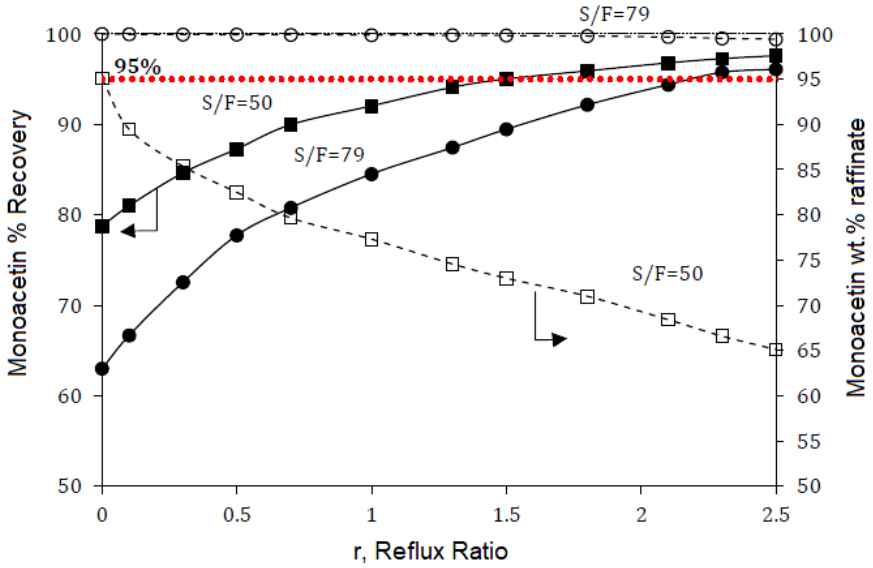
GRAPHICAL ABSTRACT



ABSTRACT

The supercritical CO_2 ($scCO_2$) fractionation can be a technically and economically feasible technology for the separation of liquid products that find application in the chemical, food, and pharmaceutical sector. Particularly, previous works shows the $scCO_2$ can be used for the fractionation of glycerol acetates, a mixture of triacetin, diacetin and monoacetin obtained by the acetylation of glycerol [1-4]. This chemical reaction is an interesting route for glycerol valorization, and it has been explored in the literature by different authors [5]. The isolation of the three compounds obtained in the reaction of acetylation is a key step in the process to get value-added products in actual biorefineries [4,5]. Previous works shows GCA-EOS model predicts accurately the phase equilibria between CO_2 and glycerol acetates mixtures [1]. Also, modeling of $scCO_2$ extractions of a commercial glycerol acetates mixture at temperatures between 301 K and 323 K and pressure between 100 bar and 130 bar shows that GCA-EOS is a useful tool to design and optimize the scale up separation process [2].

A proof of concept carried out in a bench scale high-pressure packed column (3.5 m long; 19 mm di and 373.1 kg/m^3 packing density) at 313 K and 95 bar using a solvent to feed ratio (S/F) of 17.5 g CO_2/g feed shows the technical feasibility of the fractionation process. However, the experimental results point out it is necessary to operate the column using higher solvent to feed ratio and loading conditions closer to the flooding point to decrease the height equivalent of theoretical stage [6]. Moreover, simulations of the unit show low recovery of the target compounds in a countercurrent isothermal operation. Thus, two design options are considered: i. a countercurrent column with internal reflux using a hot finger point, and ii. a countercurrent column design using an external reflux using a high-pressure pump.

The fractionation of the ternary mixture requires two columns, or a single column operated in two separation steps. In the first part, monoacetin can be obtained in the raffinate, while triacetin and diacetin can be separated in a second step. GCA-EOS modeling shows a commercial glycerol acetates mixture (23.3% g/g triacetín, 48.7% g/g diacetín y 28.0%g/g monoacetin) can be fractionated in two steps using a single column of 11 stages, fed at stage 4, and using a hot finger in the top of the column and high solvent consumption (S/F=80) to obtain 95 wt.% monoacetin at high recovery (95%). On the other hand, triacetin and diacetin can only be fractionated with an acceptable recovery of diacetin (83 %) using external reflux after recompression of the top product released from a separator at 323 K and 55 bar. Finally, to reduce solvent consumption on both columns, a thermodynamic sensitivity analysis is carried out. The more favorable operating conditions to raffinate monoacetin are 306 K and 91 bar, using a reflux ratio of 1.6 and 65 kg CO_2/kg of feed, and diacetin are 310 K and 93 bar, using a reflux ratio of 8 and 45 kg CO_2/kg Feed.

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Extraction in supercritical fluids and conventional extraction of *Baccharis tola* from northern Chile, chemical profile and enzyme inhibition

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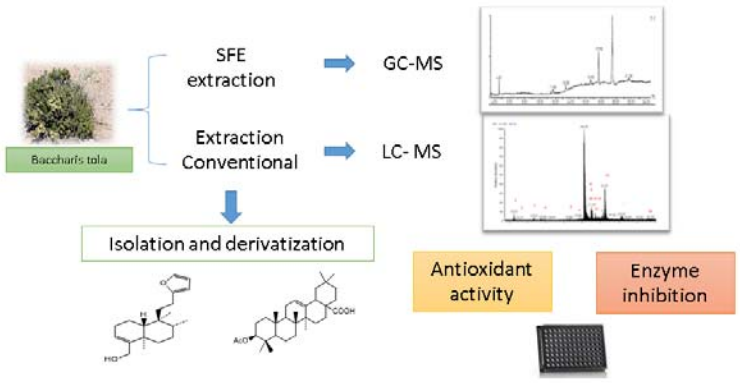
GRAPHICAL ABSTRACT



ABSTRACT

Currently, supercritical fluid extraction (SFE) is classified as one of the most used and innovative methods of clean extraction of compounds of interest from a multitude of natural resources [1]. SFE works with a pressure and temperature above the critical point of the solvent, carbon dioxide being the most widely used extractant since it has advantages such as low toxicity, non-flammable, non-explosive, high purity and low cost [2,3]. In Northern Chile, rainfall and aridity create an extreme environment for plant species that manage to develop and adapt to these climatic conditions. In this area there is a small and endemic shrub known as *Baccharis tola* belonging to the Astereacea family of which 90% is found in South America [4]. Different studies for *B. tola* showed that it has antibacterial, antifungal, anti-inflammatory, antiviral, gastroprotective and antioxidant activity [5,6,7]. Furthermore, it has been observed that the extracts of *Baccharis* species possess various terpenoids, flavonoids and coumarins [4,8]. In the present study, a comparison was made between conventional extraction and supercritical fluid extraction (Speed Helix extractor, Applied Separation, USA) of the biochemical

profile present in *B. tola*. SFE optimization was based on a 23 factorial design with two central points (10 total runs) whose study factors were: pressure (20 and 40 MPa), temperature (30 and 60ºC) and co-solvent (0 and 20% v / v ethanol) and with a constant flow of CO_2 (3.64 g / min). The optimal supercritical extraction time was set at the central point of the design (30 MPa / 45 ° C / 10% ethanol) based on the Spline Line mathematical model (TFER = 40min, ~ 90% yield). For conventional extraction, the samples are dried at 37ºC, ground and macerated in 1L hexane at room temperature for 72hrs. The extraction product is then sonicated and filtered, then brought to dryness by means of a rotating rotary evaporator at 40ºC, and stored at -20ºC, in order to then carry out the respective analyses. Then the same extraction procedure with ethyl acetate is carried out on this biomass. Subsequently, to the obtained extracts, the metabolomic profile was performed using mass spectrometry by LC-MS and the supercritical extracts by GC-MS together with the Yield, tentatively identifying the major compounds of the species.

Fractionation of the organic extracts (hexane and ethyl acetate) was carried out and a Bt1 Bacchineol diterpene, a Bt2 oleanolic acid triterpene was isolated using different chromatographic techniques and later derivatives of each compound were made to evaluate the enzymatic inhibitory activity on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The antioxidant activity of each extract was also evaluated using DPPH and ORAC bleaching experiments. Good inhibition against AChE and BChE enzymes was found for SFE extractions using ethanol as cosolvent and for compounds Bt1 and Bt2. Our findings suggest that *B. tola* is a rich source of bioactive terpenes with the potential to continue evaluating different biological activities, which is why we will evaluate the trypanocidal effect.

ACKNOWLEDGEMENTS

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Valorization of the industrial solid residue generated after agar extraction from *Gelidium sesquipedale,* by emerging technologies

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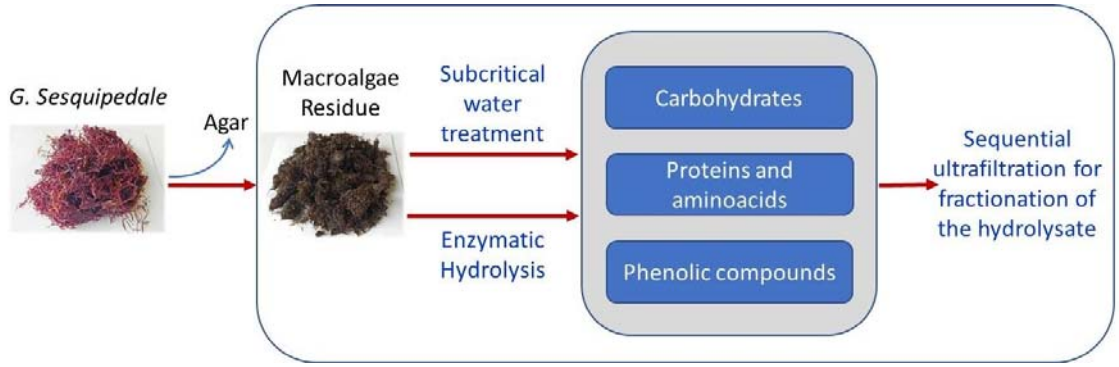
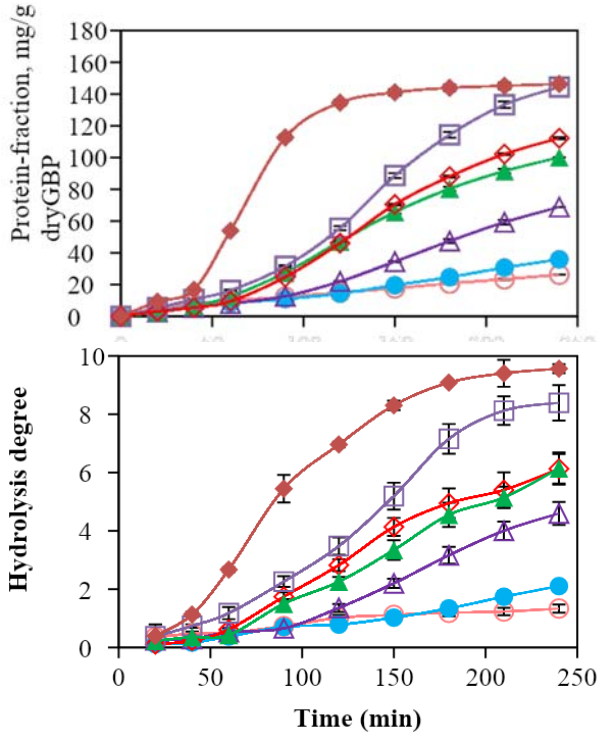
GRAPHICAL ABSTRACT



ABSTRACT

Increasing wastes as consequence of the growing population and higher quality of life, is one of the biggest problems that society faces with nowadays. The concept of biorefinery, in which residues are reincorporated into industrial processes to obtain base molecules, emerges as a solution. In this work, a complete valorization of the red alga *Gelidium sesquipedale* solid byproduct generated after industrial agar extraction (GBP) is proposed. Despite being usually discarded, it still contains high content of interesting molecules such as carbohydrates, proteins, amino acids, etc., that can be recovered.

Traditional extraction methods have several drawbacks. They are usually time-consuming and have low selectivity and extraction yields. In addition, large volumes of organic solvents, which are dangerous, too expensive and can be harmful for environment and human health, are used. As an alternative, subcritical water treatment (SWT) is proposed in this work to recover the compounds of interest in the above mentioned *Gelidium sesquipedale* byproduct (GBP). Also, enzymatic assisted extraction (EAE) has been studied in order to compare SWT with other technologies. Different hydrolytic enzymes (cellulase and protease) have been also used for this purpose. Finally, different pressure-driven membrane technologies have been evaluated for fractionation and concentration of the subcritical water extracts, with the purpose of obtaining isolated and high-purity concentrates of the extracted bioactive compounds.



The results obtained allow us to conclude that the extraction/hydrolysis taking place during SWT favor the recovery of several bioactive compounds from GBP (see Fig. 1), providing high yields through a complete byproduct valorization. SWT showed to be highly influenced by temperature, heating rate and residence time [1, 2]. SWT led to an efficient extraction/hydrolysis of the protein fraction of GBP. The best experimental conditions in a semi-continuous fix-bed reactor were 200 °C and 6 mL/min with nearly 70% of the solubilized protein content. The highest content of individual amino acids was obtained for small amino acids such as valine, alanine and glycine as well as aspartic acid. Therefore, an increase in the non-polar selectivity was observed by working at high severity factors. Positive and strong correlation was obtained between the phenolics recovered and the reducing capacity of the BP extracts. EAE has also been proven to be an efficient technology to valorize the GBP [3]. Longer times were needed and lower yields of biocompounds extraction/hydrolysis were observed for EAE in comparison with SWT. Ultrafiltration with tubular inorganic membranes has been proven to be a suitable separation technology to fractionate subcritical water extracts from macroalgae residue. The most influential parameter in separation process was the MWCO of the membrane. Further research about the functional properties of the concentrated and isolated biocompounds is needed in order to study their possible applications.

ACKNOWLEDGEMENTS

The authors acknowledge Hispanagar for kindly providing the algae materials used in this work and the funding of AEI [grant numbers PID2019-104950RB-I00 and PID2020-116716RJ-I00 / AEI / https:// doi.org/10.13039/501100011033] and JCyL and ERDF [grant number BU050P20]. E. Trigueros and P. Alonso-Riaño predoctoral contracts are funded by JCyL and ESF [ORDEN EDU/574/2018 and EDU/556/2019 respectively], A.E. Illera post-doctoral contract was funded by Junta de Castilla y León (Spain) and ERDF through project BU050P20 and R. Melgosa by a Beatriz Galindo Research Fellowship [BG20/00182].

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Subcritical water extraction scale-up from laboratory to pilot system for red algae residue valorization

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ABSTRACT

Gelidium sesquipedale solid residue after industrial agar extraction contains high amounts of proteins with all essential amino acids and carbohydrates such as glucans, galactans or arabinans [1]. Therefore, although it is generally discarded, its reincorporation in the industry as a value-added product brings an interesting solution. Traditional methods used for bioactive compounds extraction from different raw materials present numerous drawbacks, namely, time-consuming, costly to dispose of used products and harmful to environment and human health [2]. Among green technologies, subcritical water extraction (SWE) stands out as a great alternative to traditional extraction processes. SWE consists of using hot pressurized water above its boiling point, 100 °C, and below its critical point, 374 °C, which causes many of the properties of water to change, such as density or dielectric constant [3,4]. Water dielectric constant, which is related to its polarity, decreases with increasing temperature, similar to organic solvents, at 200 °C. As a result, through the dielectric constant modulation with temperature, SWE is able to selectively extract polar or non-polar compounds [5].

In order to assess the feasibility of industrial-scale subcritical solvent extraction, a pilot-scale process must first be tested. Generally, the design of the industrial SWE equipment is preceded by the study of laboratory- and pilot-scale systems. Hence, although in many cases the pilot-scale study stage is eliminated, the scaling-up process would be much more efficient by incorporating the pilot-scale study to obtain quality data and determination of scale-up factor [6]. Therefore, the main goal of this research was to prove the feasibility of industrial-scale subcritical water system through scaling-up from lab to pilot system., by the comparison of lab- and pilot-scale subcritical water performance. For this, many analytical methods were applied for the comparison of the extraction yield of the two systems; such as, polysaccharide fraction identification and quantification, total protein content and free amino acids determination, and total polyphenol content (TPC) and antioxidant activity.

Galactose was mainly recovered as oligomer fraction with maximum yields of 71.4 (36 minutes) and 74.5 % (45 minutes) for pilot and lab-scale, respectively (Figure 1a). Lower yields were determined for glucans, with maximum yields of 9.5 % for both systems, in which more than 6 % was extracted in the first minutes. Similar extraction curves and yields were determined for protein fraction with final extraction yields close to 40 % (Figure 1b), while free amino acids content was higher in laboratory scale. The greatest extraction yield was accounted for the smallest amino acids, such as glycine, alanine and aspartic acid, whereas polar amino acids such as glutamic acid and lysine were reduced, although lysine was not detected in pilot system.

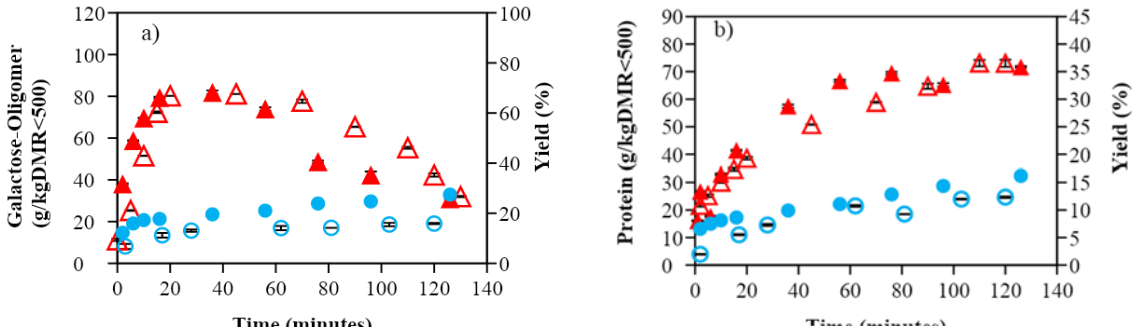

Figure 1. a) Sugars yield extraction and b) Protein yield extraction along SWE from DMR<500 for galactose as oligomer at lab-scale: 130 (○) and 175 ºC (△), and pilot-scale SWE system: 130 (●) and 175 °C (▲).

Differences in total polyphenolic compounds (TPC) extraction were observed for both systems. Increasing TPC content with time was determined for lab-scale system, while in pilot system a plateau phase was observed after 36 minutes of extraction.

SWE has been proven to be an efficient technology for bioactive compounds recovery such as carbohydrates, protein and amino acids from algae residue. Scaling up of subcritical water system from laboratory to pilot scale resulted in good and reproducible results. Therefore, feasibility of industrial-scale subcritical water system through scaling-up from lab to pilot system has been showed.

ACKNOWLEDGEMENTS

The authors acknowledge Hispanagar for kindly providing the algae materials used in this work and the funding of AEI [grant numbers PID2019-104950RB-I00 and PID2020-116716RJ-I00 / AEI / https:// doi.org/10.13039/501100011033] and JCyL and ERDF [grant number BU050P20]. E. Trigueros and P. Alonso-Riaño predoctoral contracts are funded by JCyL and ESF [ORDEN EDU/574/2018 and EDU/556/2019 respectively], A.E. Illera post-doctoral contract was funded by Junta de Castilla y León (Spain) and ERDF through project BU050P20 and R. Melgosa by a Beatriz Galindo Research Fellowship [BG20/00182]

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Compressed fluids for the sustainable valorization of winemaking by-products with antioxidant properties.

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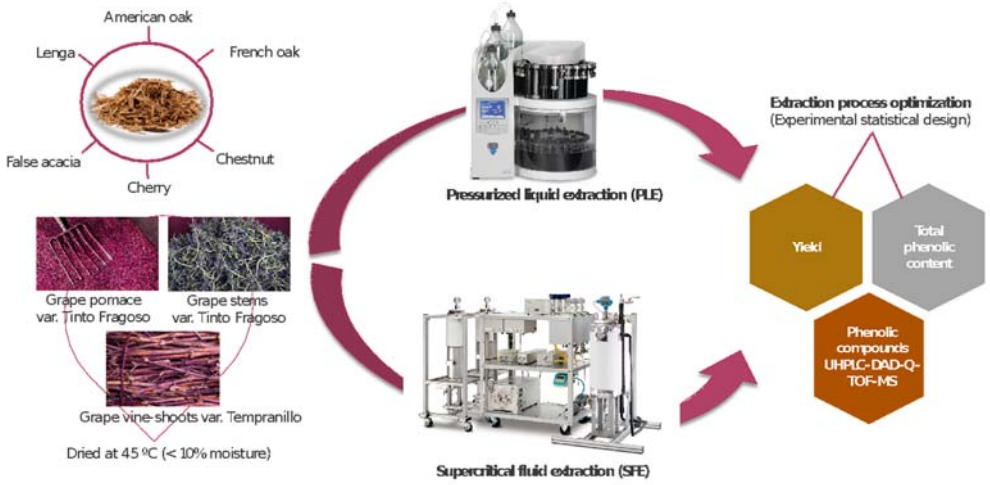
GRAPHICAL ABSTRACT



ABSTRACT

Spain is the third country with the largest wine production worldwide, generating about 33.5 million hL of wine in 2019 [1]. Apart from wine products, oenological industry also generates different kind of by-products, which entails the generation of thousands of tons of solid and liquid waste, reaching up to approximately 30% of raw material employed [2]. Grape pomace represents the main solid residue of the oenological industry (60% on average) [3]. However, a large amount of other residues is generated throughout the vinification process like vine-shoots, stems or wood chips, which come from the harvest, stemming and wine aging stages, respectively. All of the aforementioned by-products present a serious environmental and economic problems. The valorization of these matrices could be an alternative to minimize the waste accumulation and, at the same time, create innovative value-added commodities due to their high content of bioactive phenolic compounds including phenolic acids, flavonoids and stilbenes [4]. Thanks to their attractive advantages, PLE and SFE are excellent techniques to extract bioactive compounds from oenological by-products of interest for food, pharmaceutical and cosmetic industries without degrading their functional properties [5,6].

In this work, a sustainable extraction procedure for revalorization of oenological by-products were developed by comparing SFE/GLXs and PLE techniques, using

ethanol/water mixtures as green solvents. Naturally seasoned wood species chips from the wine barrels manufacturing process were used, including American oak (*Quercus alba*), French oak (*Quercus petraea*), chestnut (*Castanea sativa*), cherry (*Prunus avium*), false acacia (*Robinia pseudoacacia*), and lenga (*Nothofagus pumilio*); as well as different viticulture by-products like vine-shoots (*Vitis vinifera* var. Tempranillo), stems (*Vitis vinifera* var. Tinto Fragoso) and grape pomace (*Vitis vinifera* var. Tinto Fragoso). An optimization of the extraction process was carried out according to the yield and total phenolic content. In vitro antioxidant capacity of target extracts was also evaluated.

ACKNOWLEDGEMENTS

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Supercritical carbon dioxide extraction and enzymatic hydrolysis of brewer's spent grain

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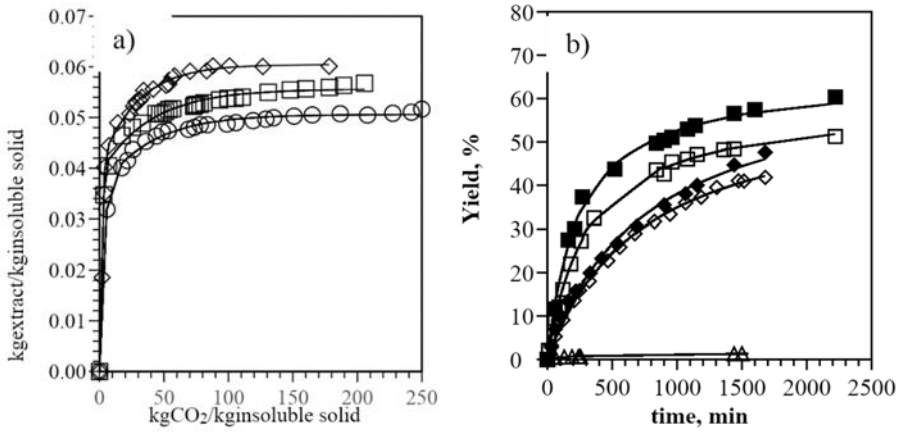
GRAPHICAL ABSTRACT



Graph. Abstract. a) Extraction kinetics of oil from BSG at 40 MPa: (○) 313 K (□) 333 K (◇) 353 K. Continuous lines represent the Sovová's model [1]. b) Glucose yield by enzymatic hydrolysis of BSG at 323 K using different cellulase dose: (△) no cellulase; (♦, ⋄) 0.5 % cellulase; (□, ■) 1 % cellulase. Open symbols: untreated BSG. Filled symbols: sc-CO_2 treated BSG. Continuous lines represent the Holtzapple's model [2], [3].

ABSTRACT

Brewer's spent grain (BSG) is one of the most important by-products in large and small-scale breweries. BSG is the solid residue generated after mashing and wort filtration process, it is generated at an average rate of 20 kg per 100 L of beer and accounts for approximately 85 % of the total residues of the brewing process [4]. Nowadays, it is mainly used for animal feed (70 %), biogas production (10 %), or landfilled [5].

However, BSG presents a valuable chemical composition with a high content of protein and carbohydrates, as well as important quantities of phenolic compounds with potential bioactive properties. BSG also contains non-negligible amount of lipids (5 %) with more than 50 % being linoleic acid (C18:2 ω-6) [6].

Due to the valuable chemical composition of BSG, different techniques have been proposed to valorize this lignocellulosic biomass, such as enzymatic and chemical hydrolysis, ultrasound assisted extraction or microwave assisted extraction [7]. High pressure processing of biomass has been also proposed since it offers unique opportunities in the extraction and valorization of the bioactive compounds of BSG.

Among the different high-pressure processes, the use of supercritical CO_2 (sc-CO_2) presents a great attractive since it is considered a green solvent and it presents gas-like (high diffusivities) and liquid-like (good solvation power) properties at supercritical conditions (T_c = 31.1 ºC p_c= 7.39 MPa). Sc-CO_2 has been extensively studied as a green extracting agent over traditional organic solvents to valorize the lipophilic fraction of biomass [8].

From a biorefinery perspective and aiming at the integral valorization of BSG, sc-CO_2 extraction and enzymatic hydrolysis processes have been applied to this by-product. First, the extraction of the lipophilic fraction of BSG with sc-CO_2 has been systematically studied. The most influential extraction conditions, namely pressure and temperature, were varied from 20 to 40 MPa and from 313 to 353 K, respectively. A maximum yield of 5.70 ± 0.07 g /100 g_{BSG} was obtained at 353 K and 40 MPa (see Graphical abstract, Fig. a)). High pressures and temperatures resulted in higher content of total phenolic and flavonoids compounds, as well as higher antioxidant capacity.

Enzymatic hydrolysis was performed on samples after sc-CO_2 treatment and non-treated BSG samples. Enzymatic hydrolysis was carried out at 323 K in an acetate buffer at pH=5 with a cellulase, 1,4-(1,3:1,4)-β-D-Glucan 4-glucanohydrolase, EC 3.2.1.4, from *Aspergillus niger* provided by Sigma-Aldrich.

The graphical abstract b) represents the glucose yield along enzymatic hydrolysis for sc-CO_2 treated and non-treated BSG at different enzyme concentrations. An improvement of the enzymatic hydrolysis yield by cellulase was observed in the sc-CO_2 treated BSG compared to the non-treated. This improvement could be partially attributed to the removal of the lipid fraction and to morphological changes of BSG after sc-CO_2. Based on this double benefit, sc-CO_2 can play an important role on biomass valorization [8].

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Lipids recovery from a new Bulgarian green algae strain *Scenedesmus obliquus* BGP by conventional and SC Extraction

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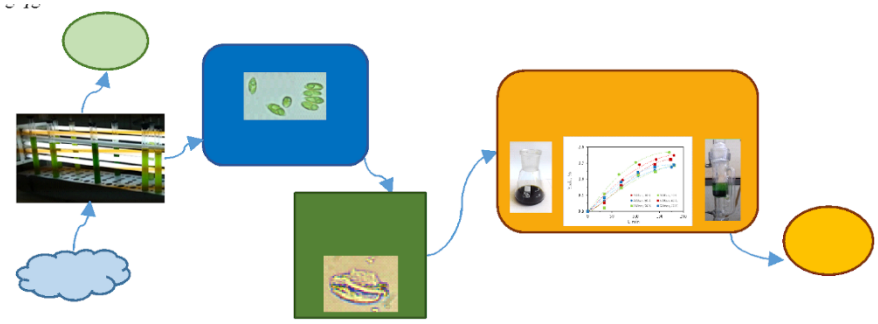
GRAPHICAL ABSTRACT



ABSTRACT

Algae show a number of important advantages as compared to conventional land plants: 10-15 times higher biomass productivities, higher CO_2 fixation rate, and sufficiency of arid or low quality agricultural land for their cultivation. Algae are advertised as a futuristic resource of bioactives, and as the most sustainable third generation feedstock for the production of bioenergy.

Scenedemus sp. is a genus of green algae that synthesizes metabolites with various properties (antioxidant, anti-inflammatory, immune stimulating, antibacterial, antifungal, antiviral, antidiabetic and anti-tumor activities), which places them among the most important producers of biologically active compounds. Recently, a new unidentified previously strain of the genus *Scenedesmus* was isolated from a rainwater puddle in Sofia, Bulgaria at an average temperature of 20 °C. The morphological analysis performed identified the new strain as *Scenedesmus obliquus* (Turpin) Kutzing [1]. The application of the Basic Local Alignment Search Tool and analysis of the phylogenetic tree revealed that the strain had the closest proximity in terms of likelihood to the *Tetradesmus obliquus* strain *Scenedesmus obliquus*. Hence, the new strain was named *Scenedesmus obliquus* BGP.

Our preliminary studies demonstrated that *S. obliquus* BGP is one of best producers of biomass and cellular compounds (proteins, carbohydrates, etc.) from its genus. Moreover, the strain shows tolerance towards variation of the most important environmental factors such as light intensity, temperature, composition of the nutrient medium. Furthermore, the lipid content of the strain is 25-30% of DW, which situates it among the best producers of lipids from *Scenedesmus* sp. reported in the literature.

In order to reveal the full potential of the *S. obliquus* BGP as a renewable biorecourse further investigations are needed. Consequently, as a first step towards that target the influence of different techniques and operating conditions on the yield and composition of the algae oils recovered from the strain biomass was examined.

To achieve the aim, firstly conventional extraction techniques (atmospheric and Soxhlet) applying solvents with different polarity were employed. Taking into consideration the rigid nature of the thick cell walls of *S. obliquus* BGP, ultrasonication was applied as a pretreatment method. In the atmospheric extractions ethanol, and methanol:chloroform (2:1) were tested. When the algae biomass was extracted *via* a boiling solution of methanol+chloroform on a reverse condenser the extraction yield (wt %) achieved was 25±4.2, while when the biomass was extracted twice with hot ethanol (1:20) under reflux the yield was 27±1.4. The yield of Soxhlet extraction with ethanol was 23.6±2.8, whereas the cumulative yield of a two-step Soxhlet (*n*-hexane followed by ethanol) was 28, namely 11.1±0.5 (step 1), and 16.9±1.6 (step 2).

In the case of supercritical extraction with neat CO_2 the following operating conditions were applied T = (40,50,60) C and p = (40, 50) MPa. The yields were much lower than that of Soxhlet with *n*-hexane, and the highest achieved was 1.86 at T= 50 C and p = 40 MPa.

Total phenolic content and antioxidant activity of the extracts were evaluated using ferric reducing ability of plasma (FRAP) assay. They were in the range (0.98-4.29) µl Trolox/g DW and depended on the operating conditions applied.

Fatty acid compositions of the algal oils extracted were analyzed by Gas Chromatography (GC with flame-ionization detector) of the methyl esters, and the peaks identification – by GC-MS detector. Oleic, palmitic, linoleic and alpha linoleic acids were detected in the highest quantities in the extracts – (28-37)%, (17-22) %, (13-16)%, (9-18)% respectively, depending on the extraction technique and solvent applied.

ACKNOWLEDGEMENTS

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Modification of brewer's spent grain after sc-CO_2 extraction: improvement of sugar and phenolic compounds release

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GRAPHICAL ABSTRACT

ABSTRACT

Brewer´s spent grain (BSG) is the solid by-product generated in breweries after the mashing and wort filtration process. It comprises about 85 % of the total by-products, generating approximately 20 kg per 100 L [1]. BSG presents a valuable chemical composition with a high content of protein and carbohydrates, 5 % of lipids and an important source of phenolic compounds. Among the different techniques proposed to valorize BSG, supercritical CO_2 (sc-CO_2) has become a promising technology to process biomass. The main aim of this work was to assess the effect of sc-CO_2, after BSG oil extraction, on the subsequent enzymatic hydrolysis to hydrolyze the polysaccharide fraction into monomeric sugars by comparing the sugar yields of sc-CO_2 and non-scCO_2 treated BSG.

After sc-CO_2 treatment at 40 MPa and 80 ºC, the raffinate obtained was subjected to enzymatic hydrolysis by cellulase at different enzyme dose. At the sc-CO_2 extraction the carbohydrate fraction remained in the raffinate phase after extraction. Glucose yield increased with enzyme concentration for non-treated and sc-CO_2 treated BSG. Higher yields of glucose were obtained for sc-CO_2 treated compared to non-treated sc-CO_2 for all the enzymes concentrations. The percentage increase in glucose yield for sc-CO_2 treated and non-treated BSG was 8, 14 and 18 % for the three cellulase concentrations essayed in this work, 0.25, 0.5 and 1 %, respectively. The hydrolysis kinetics for xylose and arabinose have been also determined for non-treated and sc-CO_2 treated BSG at 1 % of cellulase dose. Although not big differences were observed in the final sugar yield in the hydrolysates, the initial hydrolysis rate were significant higher for the sc-CO_2 treated BSG than for the untreated samples.

The higher enzymatic hydrolysis rate and yield obtained in the raffinate-BSG after sc-CO_2 treatment compared with non-treated BSG could be attributed partially to the removal of the lipid fraction. As it has been described in literature [2], fats and oils could influence the susceptibility of carbohydrates to enzymes. This improvement was also due to surface morphology modification. The structural and chemical changes of untreated and sc-CO_2 treated BSG was assessed by scanning electron microscopy and X-ray powder diffraction (XRD). Although the XRD pattern after sc-CO_2 treatment indicated that the pre-treatment was not strong enough to modify the BSG crystallinity, the raffinate exhibited an irregular porosity and lamellar structure. sc-CO_2 broke partially some structural barriers allowing a better enzyme access.

Furthermore, some phenolic compounds were determined in the enzymatic hydrolysates at the end of the hydrolysis by using 1 % of cellulase (Table 1). It was observed that for p-coumaric acid and ferulic acid, a concentration 31 and 24 % higher, respectively, was obtained in the BSG hydrolysates after sc-CO_2 treatment, while for vanillin similar concentration was obtained in both hydrolysates. The concentration of p-coumaric acid and vanillin after cellulase hydrolysis was lower than the values previously reported for the same BSG by alkaline, xylanase (1%) and subcritical water hydrolysis [3], [4]. Nevertheless, for ferulic acid, only a higher value was reached for alkaline hydrolysis.

Table 1. Phenolic compounds release yield by different treatments.

Treatment	Cumaric acid, $\mu g/g_{BSG}$	Vanillin, $\mu g/g_{BSG}$	Ferulic acid, $\mu g/g_{BSG}$	Reference
Celullase, 1 %	3.0 ± 0.3	20 ± 1	274 ± 4	This work
sc-CO_2 + Celullase, 1 %	3.9 ± 0.3	21 ± 2	341 ± 6	This work
Xilanase, 1 %	6 ± 1	111 ± 3	52.4 ± 0.9	
Alakaline hydrolysis	538 ± 4	217 ± 1	1305.7 ± 0.5	[3,4]
Subcritical water, 185 ºC	60 ± 8	330 ± 11	144 ± 10	

ACKNOWLEDGEMENTS

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Essential oil versus supercritical carbon dioxide volatile oil of needles of Aleppo pine from Tunisia

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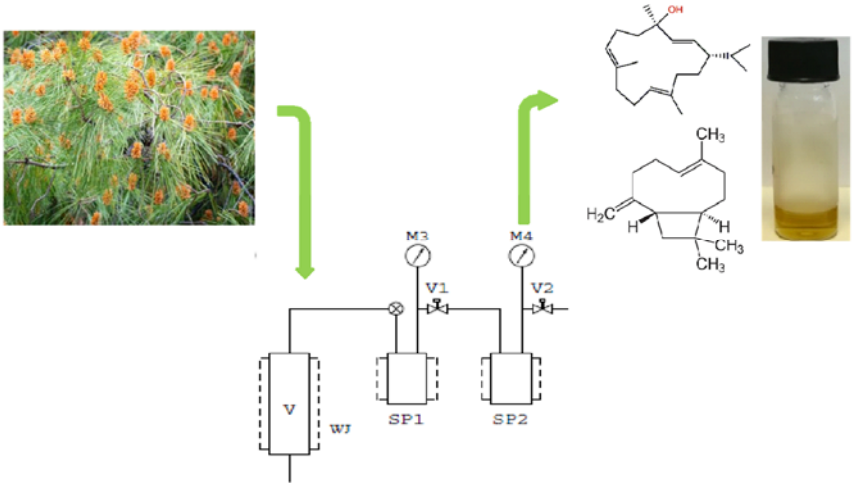
GRAPHICAL ABSTRACT



ABSTRACT

The Aleppo pine (*Pinus halepensis* Mill.) underlines itself as the first forest species of Tunisia. Despite limited production potential and unfavorable environmental conditions, the Aleppo pine forest provides multiple resources and occupies a prominent place in the forestry sector. Among the possible uses, the production of seeds for human consumption has the highest commercial value and has established itself as a priority objective in the forest economy [1]. Moreover, the needles as biomass can represent an important contribution to their valorization, from where is possible to obtain essential oil. Essential oils obtained from aromatic plants usually presents biological effects such as antibacterial, antifungal and antioxidant activities [2-4].

Needles of Aleppo pine from Ouechteta North-Est of Tunisia were collected, dried, and decreased to a mean particle size of 0.8 mm. A maximum yield of 0.8% of essential oil was obtained by hydrodistillation (HD), with a Clevenger-type apparatus, according to

the European Pharmacopoeia method. Supercritical carbon dioxide extraction (SC-CO_2) of the volatile oil from the needles of Aleppo pine was carried out in an apparatus build into the IST [5] to the pressure (90, 100 and 110 bar), temperature (40 and 50ºC), to the same mean particle size and CO_2 flow rate of 0.9 kg/h. The conditions of SC-CO2 were chosen according to previous research.

Both the essential oil (EO) and volatile oil (VO) were analyzed by GC and GC-MS and 47 components were identified, representing between 72 to 86 % of the total recovery oils. The major groups compounds were the sesquiterpene hydrocarbons (20-41%) and oxygen-containing diterpenes (21-33%). The main compounds identified were β-caryophyllene (16-31%), thunbergol (14-24%) and phenethyl isovalerate (5-9%).

The main difference between EO and VO was found to be the percentage variation of oxygen-containing diterpenes in the volatile oils (17-33%) versus the lower content in the essential oil of 21%.

ACKNOWLEDGEMENTS

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Oxindole alkaloids extraction from *Uncaria tomentosa* leaves using pressurized solvents

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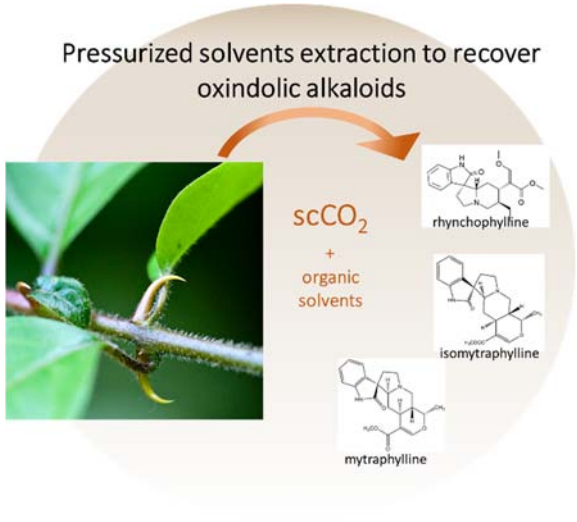
GRAPHICAL ABSTRACT



ABSTRACT

Uncaria tomentosa leaves were used in this work for selective extraction of oxindole alkaloids, which are abundant in this plant. Such compounds include rhynchophylline (RHY), mytraphylline (MYT) and isomytraphylline (IMYT).

The main aim of this work was to concentrate the alkaloids in the plant matrix, recovering the maximum of phenolic compounds and chlorophylls in the first step (pressurized liquid acetone) and to maximize the selective recovery of oxindole alkaloids using CO_2-expanded liquid ethanol, in the second step. The aim was also to evaluate, *in vitro*, the extracts' potential on the reduction of inflammation and on Alzheimer's disease.

The effect of process variables on the extraction of oxindole alkaloids was assessed. More specifically, different temperatures (313 and 333 K), pressures (16-38 MPa) and solvent densities (770-870 kg/m^3) were tested to evaluate the effect of these variables on the sequential extraction of oxindole alkaloids, by acetone and CO_2-expanded liquid ethanol.

Results showed that these alkaloids were selectively extracted in the second stage of extraction, by using CO_2-expanded ethanol. Moreover, global yield, RHY, IMYT and MYT contents were fitted by quadratic models. The analysis of variance shows that the process variables (temperature, pressure and/or density) had a significant effect on the global yield. Regarding the oxindole alkaloids contents, these variables were significant for the selectivity of IMYT and MYT, but not for RHY.

The most promising extracts were tested using *in vitro* assays with enzymes, lipoxygenase and acetylcholinesterase, and revealed potential activity against inflammation [1] and Alzheimer's disease, respectively [2]. These activities may be, however, related to compounds other than oxindole alkaloids.

ACKNOWLEDGEMENTS

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Isolation of biologically active compounds from selected natural materials with supercritical carbon dioxide

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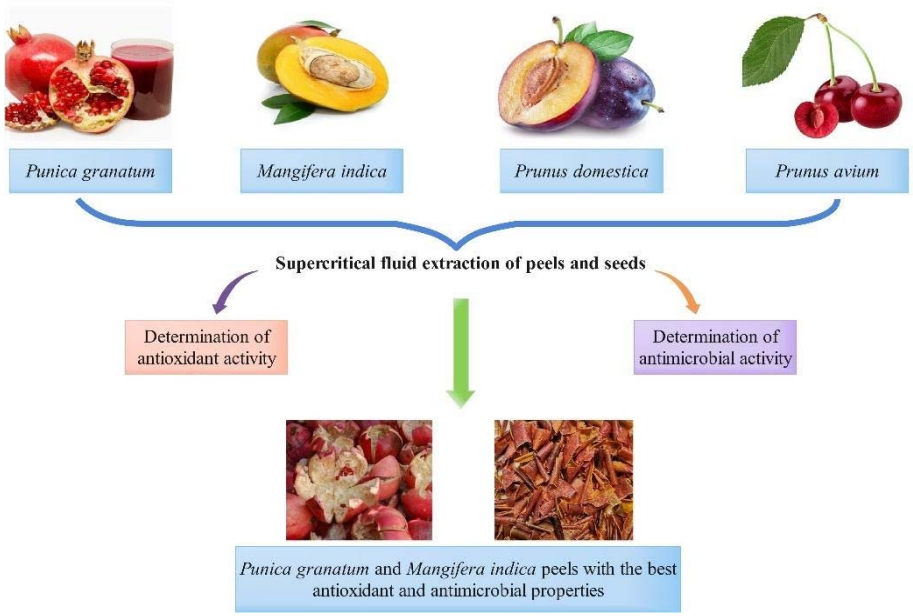
GRAPHICAL ABSTRACT



ABSTRACT

Fruit by-products from the food processing industry can have a negative impact on the environment, as they are treated as waste [1]. However, the content of biologically active compounds, including vitamins, minerals, and phenolic compounds in the peels and seeds of various fruits, is high. As a result, they exhibit good anticancer, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and many other health benefits. Therefore, the isolation of these substances is one of the most valuable approaches for the further use of fruit waste, as isolated compounds can be used in various production systems, including food, cosmetics, food, and pharmaceutical industry [2, 3]. The use of environmentally friendly extraction methods, such as extraction with supercritical fluids, is also important, as there has been growing concern in recent years about the significant impact of organic solvents on the environment and extracts [4].
The purpose of our study was to determine the antioxidant and antimicrobial properties of waste materials of selected fruit species. Fruit waste extracts such as seeds and peels

of pomegranate (*Punica granatum*), mango (*Mangifera indica*), plum (*Prunus domestica*), and cherry (*Prunus avium*) were obtained by supercritical extraction with carbon dioxide plus ethanol as a co-solvent at selected conditions at 40 °C and 200 bar. The antioxidant activity of the obtained extracts was determined using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH assay). Inhibitory properties were qualitatively determined for each extract by the Kirby-Bauer disk diffusion method on various types of microorganisms, such as Gram-positive and Gram-negative bacterial species and fungal species (yeasts and molds). The microbial growth inhibition rate at different concentrations of the obtained extracts was determined by the quantitative method of broth microdilution.

The results of the study showed that fruit waste extracts obtained with supercritical CO_2 exhibit antioxidant properties. The highest antioxidant activity was detected in mango peel extract, with an inhibition rate of 10.8%. We also proved with our study that the obtained extracts inhibit the growth of various microbial cells. The best inhibitory properties on the growth of tested microorganisms were detected in pomegranate peel and mango peel extract. Both extracts are potentially suitable growth inhibitors of Gram-positive bacteria *Staphylococcus aureus* at selected tested concentrations, as the degree of growth inhibition was higher than 90% after 12 hours of incubation. Natural extracts obtained with green extraction techniques such as supercritical extraction can significantly contribute to the medical, pharmaceutical, cosmetical, and food industries. They can be further incorporated into a variety of materials to provide antioxidant and antimicrobial properties. Also, by using waste parts of the fruit for further applications, less is discarded, which can have a positive effect on reducing environmental pollution.

ACKNOWLEDGEMENTS

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Depolymerization of lignin in SCW ultrafast process. Downstream processing simulation by aspen plus to concentrate the effluent by membranes.

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ABSTRACT

Water properties can be modulated by changes in pressure and temperature, e.g. the ionic product can be reduced by up to 10^{-18} when approaching the critical point of water. Under these conditions, it is possible to minimized reactions with ionic mechanism. Our previous results show the selectivity that can be achieved by using supercritical water (SCW) media to fractionate lignocellulosic biomass or to depolymerize lignin [1]. In our ultrafast lignin depolymerization procedure the lignin repolymerization can be minimized by the control of the residence time. In order to operate with residence time below 1 s, we use ultrafast heating by direct injection of SCW on the biomass suspension feed [2]. This heating procedure causes the reactor effluent to be very diluted. It is necessary to implement downstream processing to concentrate the effluent and recover its energy. The aim of this work is to study the effluent concentration by downstream processing simulation by aspen plus.

Sub/supercritical water technologies are among the most effective processes to produce new products with high yields, selectivity and quickness [1]. One of the disadvantages of these processes is that the product is highly diluted with water. In this work, a simulation model of the process is developed as a convenient tool for its optimization.

The downstream processing considers the next points:

1. Effluent concentration by membranes to minimize the heating dilution.
2. Heat integration to minimize the energy consume to produce the SCW
3. CAPEX reduction

A new membrane type, forward-membrane, has been considered in the simulation. This membrane can be used to separate oil from water with high efficiency, 99- 99.6 % 2. [3]. A User2 Block linked to an Excel file was used to model the membrane. In addition, a new heater was introduced to integrate the cooling energy requirement of the separator exhaust gases and the heating needs of the water feed stream (90-155 ºC). Simulation results were checked against experimental values previously obtained in our laboratory [4] [5].

The properties of components not available in Aspen Plus database were estimated using the NIST ThermoData Engine or introduced manually. The chosen Property Method was SR-POLAR which is based on an equation-of-state model by Schwarzentruber and Renon, which is an extension of the Redlich-Kwong-Soave equation of state. We can apply the SR-POLAR method to both non-polar and highly polar components, and to highly nonideal mixtures. This method is used for high temperature and pressure applications [6].

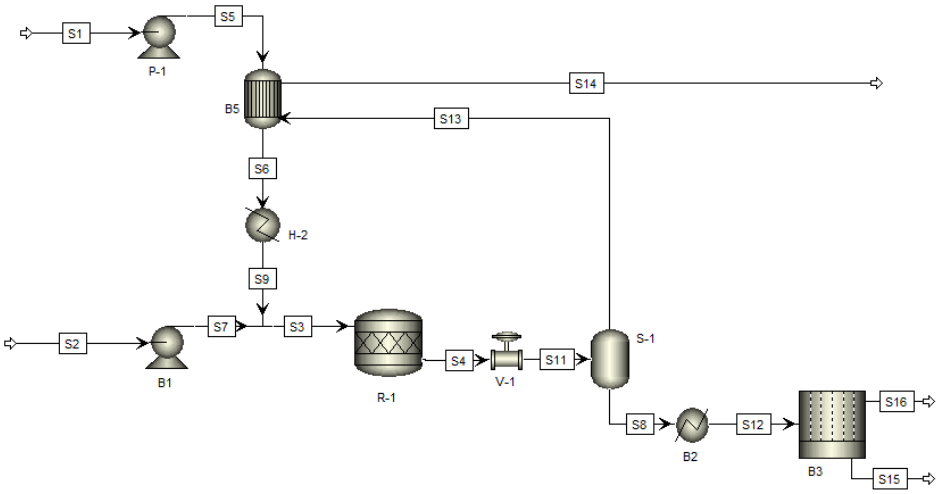

Figure 1: Simulated model on Aspen Plus

The membrane feed stream, S12, has a total mass flow of 3.2 kg/h, of which 0.127 kg/h is oil. This one is easily separated from water using the forward membrane, with a rejection of water in the range 99.0-99.6%. The amount of water reported by the simulation in the permeate (S16) is 0.0123 kg/h, about 8-10% of the total mass flow. The heat recovered from the exhaust gases (S13) in B5 allows for 67 Kw energy savings (10% of H2 heating demand), for a water feed stream (S1) of 4 kg/h. The heat removed from S8 in B2 can be used in other process sections such as the scCO2 extraction of oil from the residue in stream S16.

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Reactions

Catalytic reactions using coordination polymers in supercritical CO_2 medium: knoevenagel condensation with Zn(II) CP catalyst

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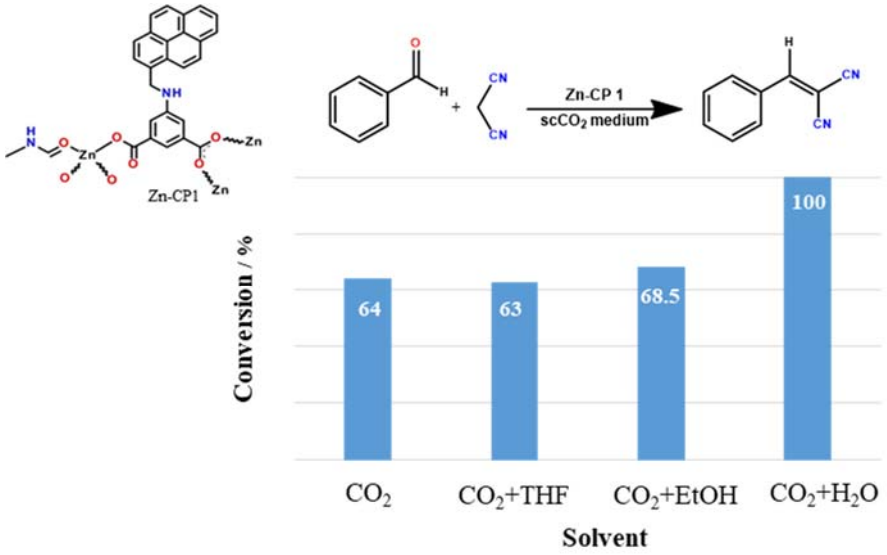
GRAPHICAL ABSTRACT



ABSTRACT

The replacement of conventional organic solvents by the so-called "green solvents" is an usual approach to more sustainable chemical processes. Carbon dioxide, due to its properties, has been successfully regarded as a common replacement when in a supercritical state ($scCO_2$). Moreover, it possesses moderate critical pressure and temperature conditions and may be easily separated from the catalytic system by a simple depressurization.

Coordination polymers (CPs) are usually described as a type of highly promising functional materials having 1D to 3D architectures. These materials have shown potential applications in a wide range of applications, from gas storage and separation, to drug delivery or catalysis [1]. Due to the insolubility in common organic solvents and

robustness, various CPs were investigated as heterogeneous catalysts for several organic transformations, such as Henry reaction, Knoevenagel condensation, oxidation of alkanes, among others [2]. Nevertheless, in most of the CPs based catalysis various organic solvents, such as MeOH, EtOH, DMF, CH_3CN, have been used [3]. For some CPs, catalysis under solvent-free conditions have shown promising results [4]. However, catalysis under $scCO_2$ using CP as catalyst has not yet been well studied.

In the present work, the potential of a new Zn(II) coordination polymer $[Zn(L1)(NMeF)]_n \cdot n(NMeF)$ (Zn-CP **1**) as catalyst was studied in the Knoevenagel condensation of benzaldehyde and malononitrile, a well-known model reaction, under very mild conditions, in $scCO_2$ medium. The effect of the experimental parameters, such as reaction temperature and time, type and quantity of co-solvent added, in the yield of the reaction was evaluated.

The studies of the catalytic system with pure CO_2, e.g. without addition of co-solvent, showed that there was no significant change in the condensation yield when the reaction time increased. On the other hand, a relevant effect of the reaction temperature on the catalytic process was observed. Concerning the experiments with the addition of co-solvent, an increasing trend was observed in the reaction yield when moving from aprotic (THF) to protic (EtOH and H_2O) polar (co-)solvents, reaching the full conversion in the latter case. It was found that $scCO_2$, in the absence of a strongly protic co-solvent, will not be the most suitable medium for this reaction. Moreover, the recycling of the catalysts through its use during three consecutive cycles showed that there was almost no loss of the catalytic activity in supercritical medium. Furthermore, SEM, PXRD and FT-IR analyses indicated a considerable stability of the catalyst throughout the process.

ACKNOWLEDGEMENTS

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High Pressure CO_2 Conversion into Methanol: A Techno-Economic Analysis of an Industrial Plant at Alvaiázere, Portugal

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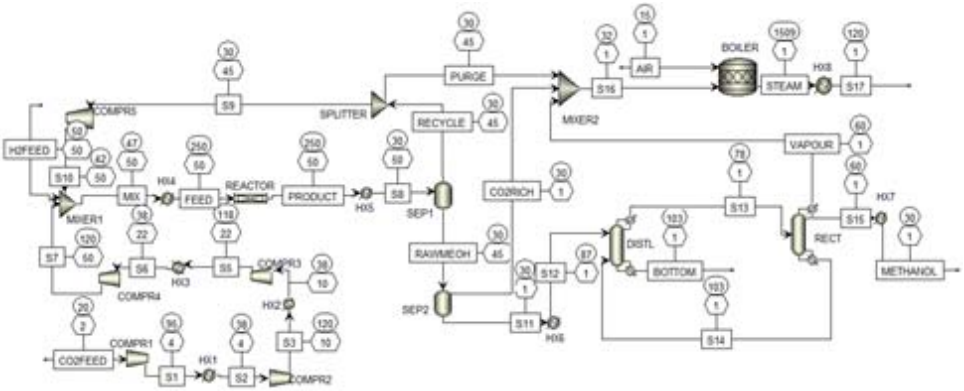
GRAPHICAL ABSTRACT



ABSTRACT

The possibility of recycling CO_2 using it as an alternative carbon source is a high interest topic, which allows not only for CO_2 valorisation, but also for the partially replacement of fossil-based feedstocks, opening the way for the so desired transition to a circular economy [1]. Indeed, CO_2 is non-flammable, non-toxic and readily available renewable C1 building block, that furthermore do not compete with food production. The field is experiencing a very fast-evolving scenario with several industrial initiatives taking place namely at pilot scales or demo size units mostly for CO_2 reduction into methanol (one of the preferable routes) [2].

Methanol is a particularly interesting application because its physicochemical properties enable an easy handling, store and transport using existing technologies [3]. Furthermore, methanol can be used as a fuel additive in conventional engines, or pure in modified engines and direct methanol fuel cells. In fact, methanol is believed to be the key for a feasible energy transition because it can be integrated in the existing energy flows and infrastructures [3]. However, the only way CO_2 can be reduced into methanol, without emitting more carbon into the atmosphere, is by using green H_2 produced from the electrolysis of water, by means of renewable energies [3]. Several techno-economic studies have been published pointing out the plant capacity, the carbon taxes and the price

of green H_2/renewable electricity as being critical points for the economic feasibility of the process [2,4,5].

In this work, the software Aspen Plus was used to carry out a process simulation of a high-pressure industrial plant located in Alvaiázeres, Portugal. Two different plant capacities for methanol production (8 kt/year and 21 kt/year) were compared by means of economic analysis with the option of buying the hydrogen or producing it directly on alkaline electrolysis plant attached to the methanol plant. Finally, a sensitivity analysis has been performed in order to find out the more decisive parameter affecting the viability of the process.

ACKNOWLEDGEMENTS
This work was supported by Fundação para a Ciência e Tecnologia FCT/MCTES (project PTDC/EQU-EPQ/31926/2017), and by the Associate Laboratory for Green Chemistry – LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020)

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Thermodynamics, Phase Equilibria, and Transport phenomena/properties

Supercritical CO_2 injection into oil reservoirs: Effects of phase density changes on the recovery factor

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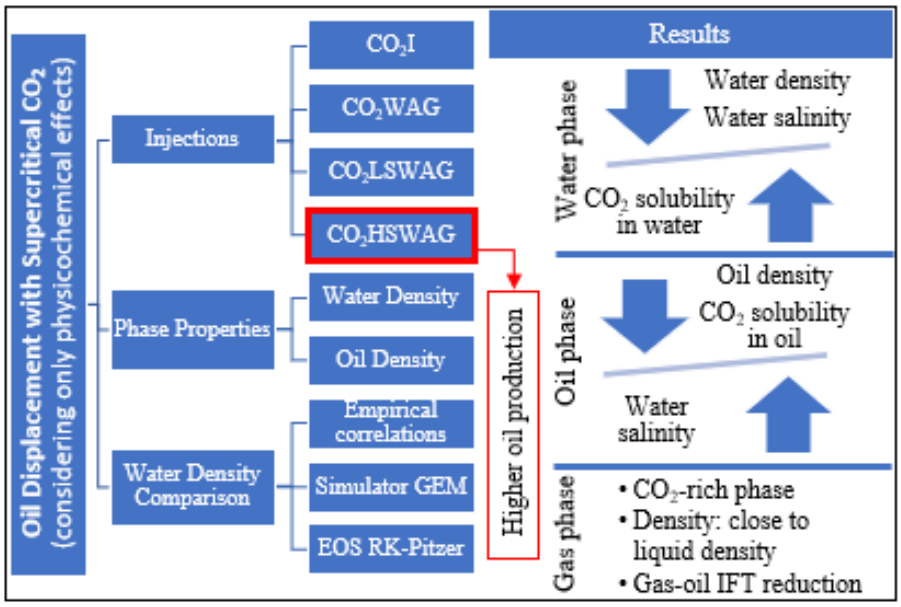
GRAPHICAL ABSTRACT



ABSTRACT

One of the current applications of supercritical CO_2 ($scCO_2$) is its injection into oil reservoirs for the improvement of oil recovery, which also contributes to the mitigation of this greenhouse gas effect. Besides CO_2 continuous injection (CO_2I), some alternating fluids schemes have been investigated, such as water-alternating-CO_2 (CO_2WAG), low-salinity water-alternating-CO_2 (CO_2LSWAG) and high-salinity water-alternating-CO_2 (CO_2HSWAG), aiming to optimize operational conditions. Monitoring reservoir conditions allow an adequate understanding of $scCO_2$ interactions with the fluid phases (oil, gas and water) and to evaluate which properties mostly affect the fluids flow in the porous medium as well as the oil production. For instance, higher CO_2 solubility in the oil phase implicates higher oil density [1] due to the CO_2 ability to vaporize light components from the oil, which favors the oil recovery. Oil displacement and recovery can also increase due to the salting-out effect, when brine salinity increases and more CO_2 is available for solubilization in oil. In this context, the use of $scCO_2$ for oil recovery is also encouraged due to its high density and high diffusivity, which increases the CO_2 fraction in the oil. This study focuses on the evaluation of the density effect on the recovery factor during oil displacement through CO_2I, CO_2WAG, CO_2LSWAG and CO_2HSWAG injection techniques. Geochemical effects on the oil recovery factor due to reactions between fluids

and the reservoir rock were neglected in this preliminary evaluation but rock dissolution and ion exchange are possible mechanisms reported in the literature. With oil and reservoir data from the literature [2], simulation of oil displacement for these 4 injection techniques was performed using the Peng-Robinson equation of state and the GEM compositional reservoir simulator (CMG®), aiming to monitor reservoir phases properties and to determine density effects. For CO_2LSWAG, a salinity of 5,000 ppm was considered while for CO_2HSWAG it was 50,000 ppm. Reservoir temperature was 71.11ºC, initial pressure was 55158.07 kPa and an injection process time of 5 years was simulated. As an initial step, 8 empirical correlations to calculate the density of the water phase (water and dissolved NaCl and CO_2) were evaluated using experimental data. GEM uses a correlation to calculate the water density, which was compared to the expected values provided by a phenomenological model (relative deviation of 3.9%) and the two most accurate empirical correlations (relative deviation of 0.07 and 0.18%). Besides monitoring properties over simulation of the aforementioned injection techniques, the density effect on oil production estimates was investigated.

As the relative deviations for the empirical correlations are the lowest, the correlations are more appropriate than the phenomenological and GEM ones, because GEM aqueous density estimates were much closer to the phenomenological model. Although results by GEM are fine, there are other models in the literature more accurate and simpler to use. Results indicate that the highest oil production was obtained with CO_2HSWAG and the lowest was obtained with CO_2I, meaning a difference in financial income of, approximately, 8000 oil barrels. This is due to the smaller difference between the oil and gas densities (difference of 347 kg/m^3 for CO_2HSWAG and 462 kg/m^3 for CO_2I) and between oil and water densities (difference of 429 kg/m^3 for CO_2HSWAG and 533 kg/m^3 for CO_2I) at the last year of the simulation. As a consequence, lower interfacial tension values are reached for CO_2HSWAG, which facilitates oil displacement and increases oil recovery. The oil phase density remains close for both injections. The density difference for the injections occurs due to the different water salinity and the distinct amount of CO_2 that dissolves on water and oil phases, modifying oil recovery. Furthermore, for CO_2LSWAG and CO_2WAG, results for oil density are nearly the same (630.08 and 630.25 kg/m^3, respectively), which means that the increase in water salinity, due to LSW injection, was not enough to further increase oil phase density. It is noteworthy that the gas phase density increases, being closer to the liquid phase density at the end of the simulation, as expected. As the oil production calculated for the methods was not quite different, it is assumed that the physical mechanisms do not represent the controlling step of oil recovery.

ACKNOWLEDGEMENTS

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Measurement and modeling of the density of the mixture scCO₂ + CH₄

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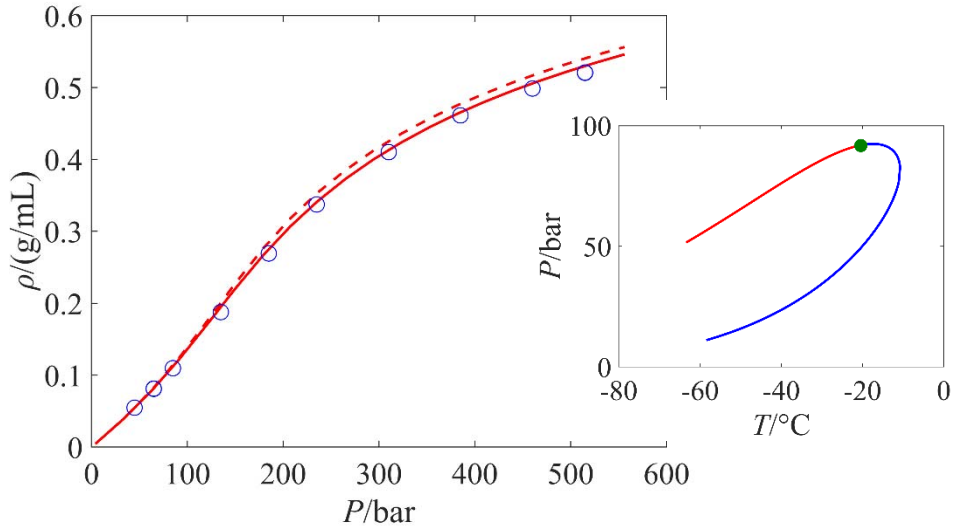
GRAPHICAL ABSTRACT



ABSTRACT

Supercritical fluid injection into petroleum reservoirs is among the methods to improve oil production [1], which is typically performed using CO_2, N_2 and associated gas [2]. In the Brazilian Pre-Salt fields, a gas composed mainly of CO_2 and CH_4 is available for injection into the reservoirs. Donnelly and Katz [3] measured the critical point of this binary mixture in different proportions, reporting a maximum critical pressure of 86.2 bar (29.5% of CH_4) and a maximum temperature of 31 ºC (pure CO_2). As the Pre-Salt reservoir pressure and temperature conditions (555 bar and 61 ºC) are much higher than these values, it is expected that the injection fluid is in the supercritical state. Seitz *et al.* [4] measured the density of the CO_2 and CH_4 mixture, including data in supercritical conditions. However, the conditions studied are not quite those encountered in the Pre-Salt fields, which is the main motivation of the present study. The injection fluid studied in this work is a 50%-50% molar CO_2+CH_4 mixture. As the properties of this mixture in supercritical conditions have not been reported in the literature yet, we carried out experiments to measure the density.

The knowledge of the injection fluid density has crucial importance for engineering calculations, affecting gravity segregation of fluids present in the reservoir and their

interfacial tension, ultimately impacting the prediction of oil production. In this work, we used a high-pressure densimeter (Anton Paar DMA HPM) to measure the density of the CO_2 and CH_4 mixture at supercritical conditions. This densimeter computes the period of oscillation of a U-tube filled with the fluid. This period is correlated with the density by performing a calibration procedure: the period of oscillation of reference fluids is measured and used to construct a calibrating function. The density of the fluid of interest must be between the density of the reference fluids. Initially, we chose N_2 and water as reference fluids and used the LAB Fit software [5] to regress the data using over 200 functions of its database of up to four adjustable parameters. However, none of these functions were able to describe the data satisfactorily. We believe that the huge differences between the fluids' density and phase behavior lead to such a discrepancy in the measured data that a simple function with a few parameters cannot describe it. More specifically, water is liquid at all pressures in the measured conditions, but N_2 suffers a phase change, from gas to supercritical fluid. We then replaced water with CO_2, which has the same phase behavior of N_2 in the measured conditions and a density higher than the CO_2-CH_4 mixture and were able to successfully calibrate the equipment, obtaining an average experimental error of 0.2% and a maximum experimental error of 0.6%.

The measured densities are presented in the main graphical abstract. All measurements were taken at 61 ºC. The PC-SAFT equation-of-state [6] was used to describe this property data as a function of pressure. A reasonable prediction (average relative deviation of 3.80%) is obtained without adjusting any parameter (i.e., setting the binary interaction parameter to zero), shown in the dashed line. The description is improved (average relative deviation of 1.58%) using literature data [4] to adjust the binary interaction parameter (solid line). The data measured in this work were not used in this adjustment to show that they are in good agreement with the prediction of a state-of-the-art model. This model was also employed to obtain the phase envelope of the mixture, shown in the inset of the graphical abstract. The calculated critical pressure of the mixture is approximately 91 bar, qualitatively agreeing with the data by Donnelly and Katz [3], who reported 84.5 bar for the mixture with 45.7% of CH_4. The model correctly predicts an increase in critical pressure, when compared with the pure components. If desired, this prediction could be severely improved using phase equilibrium data.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Agência Nacional de Petróleo, Gás Natural e Biocombustíveis (ANP) and the Petrogal Brasil S.A. for the financial support.

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Models for tracer diffusivities in liquid and supercritical ternary systems

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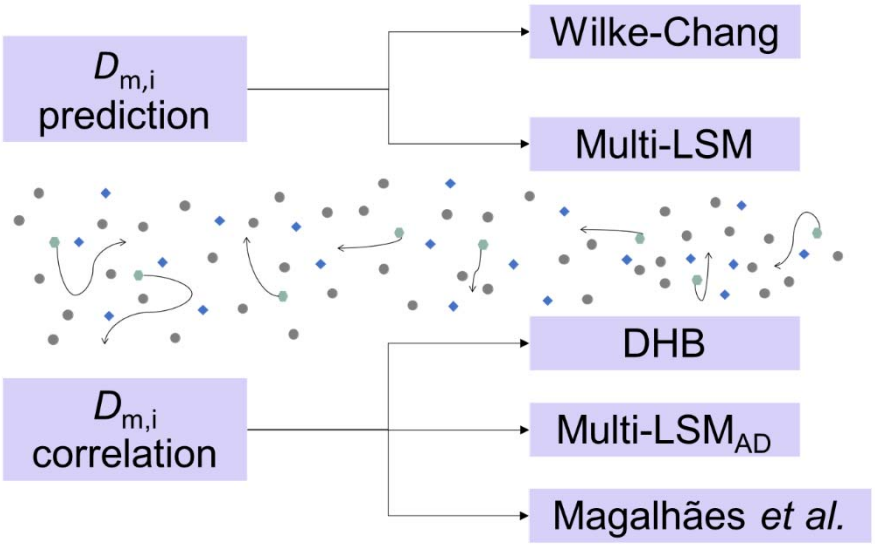
GRAPHICAL ABSTRACT



ABSTRACT

Molecular diffusion coefficient (D) is a transport property of chief importance for accurate equipment design and modeling of processes involving mass transfer. Among them one can cite supercritical fluid extraction (SFE) where the use of supercritical CO_2 is commonly used, modified or not with a cosolvent [1]. Therefore, this may require not only binary diffusion coefficients (D_{12}) (*i.e.*, a solute in a pure solvent) but also multicomponent diffusion coefficients ($D_{m,i}$) (*i.e.*, a *solute* in a solvent mixture) [2]. However, experimental data for multicomponent systems at several experimental conditions (*e.g.*, temperature, pressure and cosolvent fraction) are limited, which is consequence of the costly equipment and analytical standards required for $D_{m,i}$ measurement. Hence, accurate predictive models and correlations for $D_{m,i}$ calculation are needed [3].
The main goal of this communication is to discuss the best models available for $D_{m,i}$ estimation and correlation, as well as to develop a new model for this purpose. Accordingly, it was compiled the largest database ever of ternary diffusivities (a trace solute in a solvent mixture of 2 components) consisting of 1530 data points from 153 supercritical and liquid systems. From these, 40 systems correspond to CO_2 modified with

a cosolvent (53 % of the data points total) and 113 systems correspond to organic liquid mixtures (47 % of the data points total). Then, using these data, several models and correlations were tested, namely, the Wilke-Chang [4] equation, the newly developed Multi Liu-Silva-Macedo (Multi-LSM) model, the 2-parameter correlation of Dymond–Hildebrand–Batschinski (DHB) [1,5], four of the simple 2-parameter correlations of Magalhães *et al.* [6], and the new 1-parameter correlation variant of the Multi-LSM denominated Multi-LSM_{AD}. Overall, all models show very good performance with the Wilke-Chang equation achieving an average absolute relative deviation (AARD) of 12 % and the Multi-LSM an AARD of 10 %. Regarding the correlations, the 1-parameter Multi-LSM_{AD} achieves AARD = 4 % while the remaining 2-parameters correlations achieved AARD values between 3 % and 8 %.
All models and correlations were found to estimate very satisfactory $D_{m,i}$ values, with the final choice being tight to the systems properties known. In the whole, the Wilke-Chang model is recommended due to its simplicity.

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Experimental determination of phase equilibria at high pressures of the ethyl esters of palm oil and murumuru oil ethyl esters in carbon dioxide

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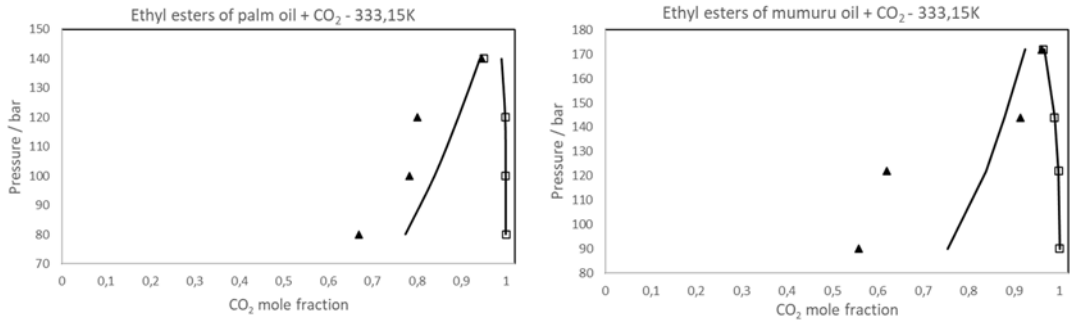
GRAPHICAL ABSTRACT



Composition of Ethyl Esters of Palm Oil and Murumuru Oil				
Esters	M (g/gmol)	RF	Palm Oil (% mass)	Murumuru Oil (% mass)
Ethyl Laurate	228,38	1,15	0,21	51,64
Ethyl Myristate	256,42	1,18	1	35,38
Ethyl Palmitate	284,48	1,21	39,8	--
Ethyl Stearate	312	1,23	6,2	3,07
Ethyl Oleate	310	1,24	41,89	7,39
Ethyl Linoleate	308	1,39	10,89	2,52

ABSTRACT

The knowledge of the behavior of the phases is essential for the design of any process using the technology with supercritical fluids [1]. The processing of vegetable oils with supercritical CO_2 is attracting considerable attention mainly from the food, but also the pharmaceutical, nutraceutical, and biodiesel industries. The extraction of vegetable oils, the selective extraction of valuable ingredients, and the fractionation of fatty acids and fatty acid esters are now well-studied processes [2,3]. Biodiesel is usually produced from vegetable oils by the transesterification of triglycerides using an excess of methanol or ethanol. Recently, several studies investigated the potential of using supercritical CO_2 as a co-solvent or as a reaction medium in transesterification reactions [2-5].

The principal objective of this work was to carry out experimental measurements of data of gas-liquid equilibrium of multicomponent systems of ethyl esters of palm oil (Elaeis guineensis) and the oil murumuru (Astrocaryum murumuru) with carbon dioxide at pressures between 80 and 172 bar and temperature of 333.15 K, followed by determination of the composition for each condition of equilibrium by gas chromatography, thus helping to generate/provide the basis for the systematic study of the reaction of transesterification of vegetable oils, with the implementation of supercritical technology for the production of biodiesel with the use of high-value products such as concentrates of tocopherols and carotenoids. To do this was assembled a unit of phases equilibria, using the static method in the Laboratory of Chemical Engineering, Department of Thermal Separation Processes of the Technical University of Hamburg (TUHH) in Germany. After this, was available the capacity of the equation of Peng-Robinson, with the mixing rules of Van de Waals with two binary interaction parameters, to predict the equilibrium liquid-gas of pseudo-binary systems, and experimental data of this work was. The apparatus and the experimental procedure were validated with the reproduction of the data of liquid/gas equilibrium from the binary system methyl oleate/carbon dioxide at 52 and 150 bar and 333.15 K in the published literature. For the conditions of equilibrium of the different systems studied it was found that the equation of Peng-Robinson showed good results for the phase gas to both systems.

ACKNOWLEDGEMENTS

The authors thank the Department of Thermal Separation Process Engineering, Hamburg University of Technology, Germany, for the utilization of the phase equilibrium equipment.

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Measurements and correlation of interfacial tension of water in its saturated vapor and nitrogen atmosphere at high pressure and high temperature

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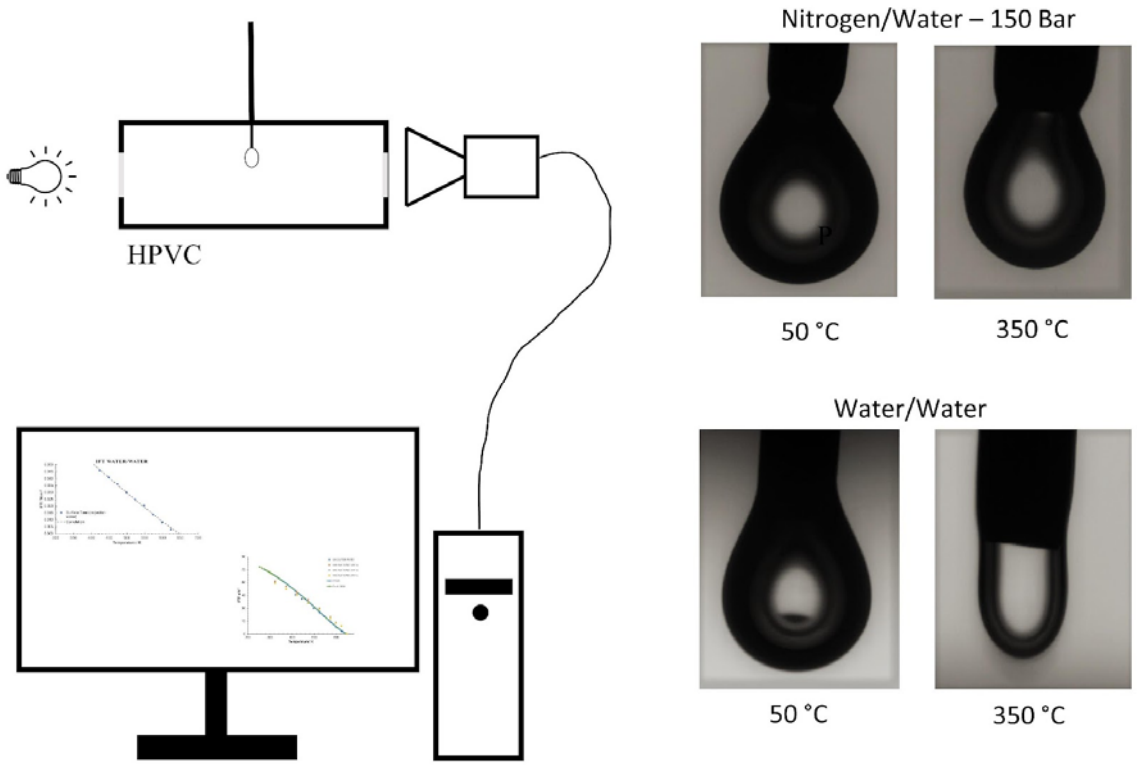
GRAPHICAL ABSTRACT



ABSTRACT

Water is widely used fluid in industrial processes due to its physical properties and benign character. The knowledge of water properties is of great importance for designing processes that use water as a reaction medium. This work discusses measurements of water interfacial tension in equilibrium with its saturated vapor and in a nitrogen atmosphere.

The interfacial tension was measured by the pendant drop method using a high-pressure visual cell (HPVC) from Eurotechncia GmbH, Germany. The drop was recorded using a

CCD camera and the evaluation of the drop shape was carried out by a drop-shape analysis commercially available from Krüss GmbH (Hamburg, Germany).

The values of water interfacial tension, up to the temperature close to the critical point, are already provided from the former International Association for the Properties of Water and Steam [1] and are based on the data that were collected and correlated by Vargaftik et al. [2]. The data provided by Vergaftik et al. is a summary of the work that has been done on the measurements of surface tension of water at low and high temperatures from various research groups, a detailed description of the measurement procedure is not available in the literature. Moreover, those data are mainly obtained using different experimental methods, among which the capillary rise method is the most reported one.

The following work was planned to provide the detailed experimental procedure of surface tension measurements using the pendant drop method and the fit of the data with previously reported results. The interfacial tension between nitrogen and water at high temperatures and pressures is also measured and the influence of nitrogen pressure on interfacial tension is discussed using empirical and thermodynamical models.

ACKNOWLEDGEMENTS
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Modelling and scaling-up of a continuous SFEE process

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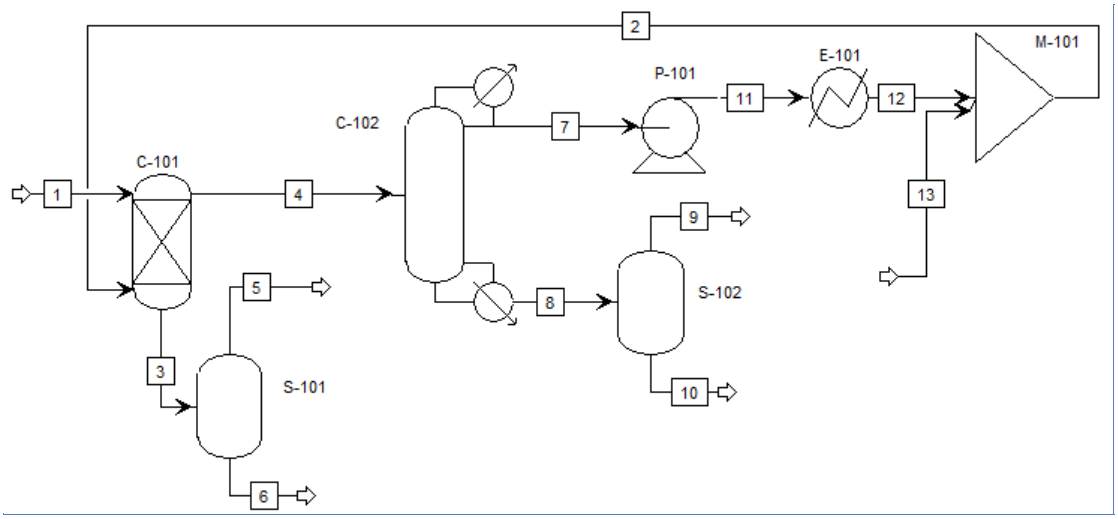
GRAPHICAL ABSTRACT



Flowsheet of the SFEE process.

ABSTRACT

In Supercritical Fluid Extraction of Emulsions (SFEE), supercritical CO_2 is used to rapidly extract the organic phase of an emulsion (O/W). When the solvent is removed by the CO_2, the substances that were dissolved in the O precipitate in small particles that remain suspended in the water, stabilized, and dispersed by a surfactant.

The process can be carried out continuously in counter-current flow of the emulsion and the CO_2 on packed columns at moderate pressure (8 MPa - 10 MPa) and temperature (310 K - 313 K). A critical stage of this technology to be economically and environmentally feasible on a commercial scale, is the separation of the organic solvent from the CO_2 for the recirculation of both streams to the process.

On the other hand, at least a ternary mixture is involved in the phase equilibrium pertinent to the SFEE column, not considering the solutes, which are normally in small concentration. The description of such complex and non-ideal mixtures at high pressure requires advanced thermodynamic models, based on equations of state with activity coefficients. The first aim of this work was to fit experimental equilibrium data with proper models for a mixture relevant for particle formation by SFEE in the temperature and pressure conditions suitable for this technology. Specifically, ternary mixtures of ethyl acetate/water and CO_2 were studied. SR-Polar correctly predicted the type II behaviour of the mixture that occurs at 8.5 MPa and 310 K, with an error for the vapour phase of less than 5 % over the entire ethyl acetate concentration range.

With this suitable thermodynamic model, the simulation of the whole integrated process was carried out using Aspen Plus®. The SFEE column and the solvent recovery separator were sized to achieve a specified amount of residual organic solvent in the leaving streams, according to the tight requirements of the food and pharmaceutical industries. For example, for an emulsion of 20 % ethyl acetate in water, it was necessary to employ only 2 equilibrium stages adiabatic column using a solvent-to-feed ratio of 10 (kg/kg), to reduce the amount of ethyl acetate in the water-rich raffinate to less than 50 ppm, operating at 310 K and 10 MPa. This would mean a column of about 1 m height, shown as C-101 in graphical abstract.

Further, the recirculation line of the CO_2 to the SFEE column was designed. Ethyl acetate is quite soluble in CO_2 even at low pressures. So that for its complete separation, it was not enough to reduce the pressure in a flash tank to 5 MPa - 6 MPa, as it is done in the recovery of "heavy" extracts in supercritical extraction processes. Instead, a distillation column simulated in Aspen Plus® as a RadFrac block (C-102) with six stages was required; reflux ratio was 1 and distillate to feed ratio was 0.85. The extract stream (4) is fed to this column to obtain ethyl acetate (8) and to recover pure CO_2 (7).

The recovered CO_2 is compressed (11) in the pump P-101 up to 8.0 MPa and its temperature is conditioned up to 38 °C (12) in a heat exchanger (E-101). To make-up the lost CO_2 from raffinate and extract, a pure CO_2 stream (13) must be fed, which is then combined with stream 12 in a mixer (M-101) to recirculate the resultant stream (2) as feed to the extraction column (C-101). The stream 8 obtained from C-102 is divided into two streams in a separator (S-102). One of the streams (10) was obtained with roughly 83 % purity of ethyl acetate which is practically free of CO_2, so that it can be used directly in the formulation of the starting emulsion (1).

Finally, the process was scaled up ten and one hundred times for increasing production capacities, given the importance of economy of scale in supercritical CO_2 installations. Operating expenses and capital expenditures were estimated. For example, for a 42 kg/h production, the installation would cost 2.5 million € while utilities would be 40.3 € (as estimated by Aspen Plus Economic Analyzer (APEA) tool.

This work could ease the technology transfer of the SFEE procedure to the industry.

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Materials, Biomaterials and Sterilization

Effect of supercritical CO_2 sterilization process on the physicochemical properties of bio-based aerogels

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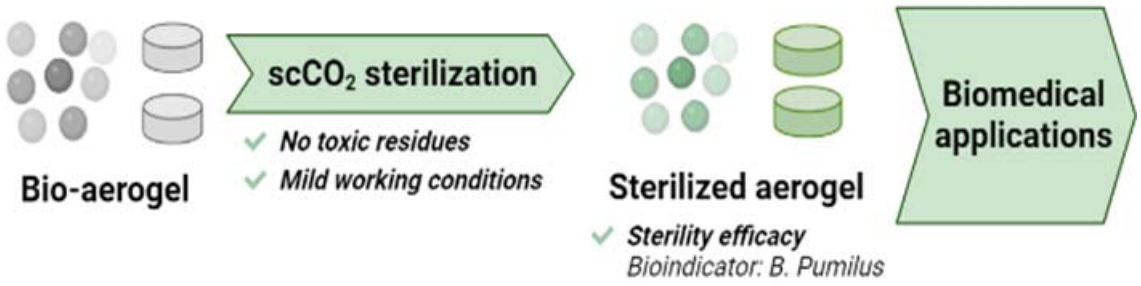
GRAPHICAL ABSTRACT



ABSTRACT

Aerogels are lightweight materials produced from many sources with customized morphologies (monoliths, beads, micro/nanoparticles, among others). Due to their low density, high specific surface area, and highly open porosity, aerogels are promising candidates for a wide range of applications. Namely, bioaerogels (made of polysaccharides or proteins) are particularly interesting for biomedical applications due to their bioactivity, biocompatibility, and biodegradability. For this application, the sterility of the materials must be ensured to avoid clinical complications linked with infections.

Conventional sterilization techniques show important limitations for the treatment of nanostructured materials as aerogels in terms of physicochemical integrity of the material and penetration capacity [1,2]. Supercritical carbon dioxide ($scCO_2$) sterilization is an effective alternative to tackle the abovementioned limitations, which operates at mild working conditions and results in sterile materials in the absence of toxic residues [3]. The pathway of cell inactivation with $scCO_2$ consists of extracellular and cytoplasmic acidification and modification of the cell membrane by extracting membrane lipids [2]. In this work, the supercritical sterilization process is tested for its efficacy in the treatment of bioaerogels of different sources and morphologies.

Starch aerogel monoliths, alginate aerogel particles and 3D-printed methylcellulose aerogels were manufactured following reported protocols intended for the biomedical field. Then, aerogels were subjected to a supercritical sterilization protocol (39ºC, 140

bar, 150 min). Commercial spore strips of *Bacillus pumilus* with 10^6 spores were used as biological indicator to evaluate the sterilization efficacy. Microbiological evaluation was performed by culturing the spores after the supercritical treatment in a trypticase soy agar culture medium. The physicochemical properties of aerogels were characterized before and after the sterilization process by scanning electron microscopy (SEM), N_2 adsorption-desorption test and gas pycnometry to evaluate any relevant change in the nanostructure. Preliminary results show that the behavior of the bioaerogels after being subjected to $scCO_2$ sterilization conditions differed on the polysaccharide source with changes in their physicochemical properties of varying extent.

ACKNOWLEDGEMENTS

Work supported by MICINN [PID2020-120010RB-I00], Xunta de Galicia [ED431C 2020/17], Xunta de Galicia-GAIN [Ignicia Programme 2021, ECOBONE], Agencia Estatal de Investigación [AEI] and FEDER funds. A.I.-M. acknowledges to Xunta de Galicia for her predoctoral research fellowship [ED481A-2020/104]. Work carried out in the framework of COST Action CA18125 "Advanced Engineering and Research of aeroGels for Environment and Life Sciences" (AERoGELS), funded by the European Commission.

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Use of polylactic acid impregnated with grape pomace extract by supercritical CO_2 for biomedical applications

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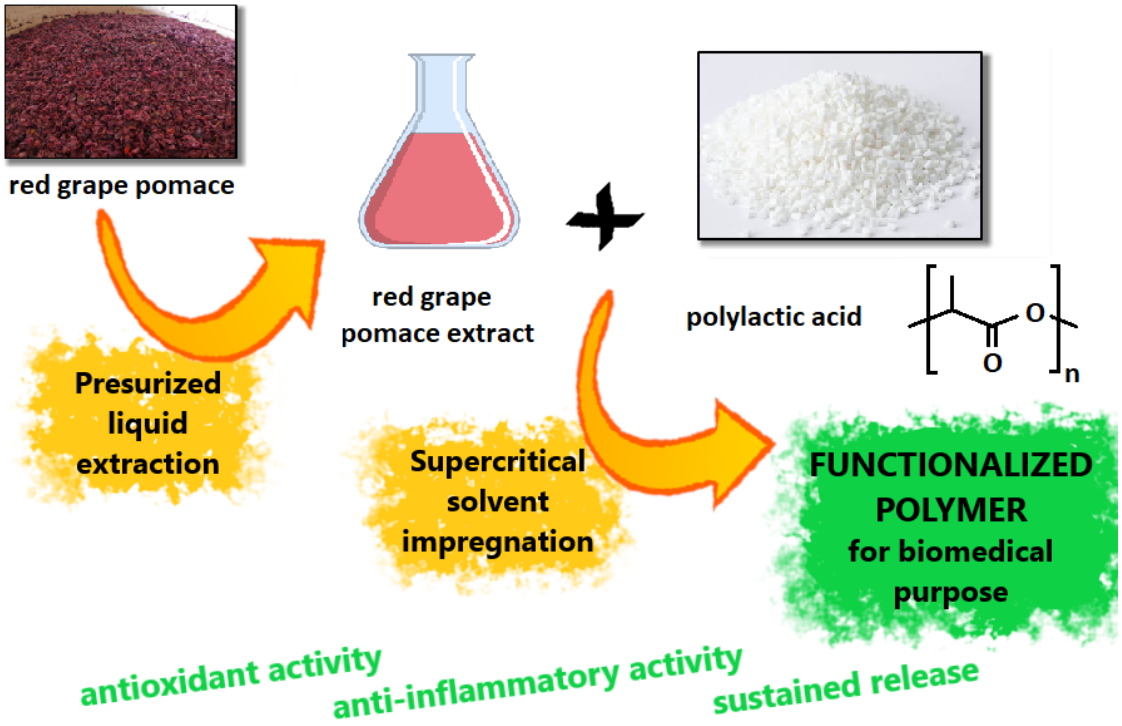
GRAPHICAL ABSTRACT



ABSTRACT

The use of polymers loaded with active principles is increasingly widespread, mainly for the dosage of drugs either in oral systems or in medicalized implants [1]. There is a current trend to replace or complement traditional drugs with natural substances that provide therapeutic benefits [2]. In this sense, it is worth highlighting the use of substances with high added value recovered from by-products of the agri-food industry.

Grape by-products (skin, seeds, pomace, stems…) have been employed in a wide range of applications such as food ingredient, cosmetics, pharmacology, and biomedicine [3-5]. Especially those from red varieties, have a high content in polyphenols and anthocyanins given antioxidant, anti-inflammatory, and anti-microbiological properties, among others [6].

In this work, a grape pomace extract from Tempranillo variety was used to functionalize polylactic acid (PLA), one of the most used polymers in drug delivery systems. First, two

different extracts were obtained using pressurized liquid extraction (PLE) in batch mode, at 250 bar and 55 ºC [7, 8]. Ethanol was the solvent for the first extract and a mixture of water and ethanol (1:1 v/v) for the second extract. These extracts were chemically characterized, and their antioxidant and anti-inflammatory capabilities were analyzed.

Then, both extracts were used to functionalize PLA by supercritical solvent impregnation (SSI), evaluating the best operating pressure and temperature condition to obtain a higher substance loading, between 100 to 400 bar and 35 to 55 ºC.

It was studied whether the impregnated polymer maintained the bioactive properties of the extract, which would make it an interesting material for use in biomedicine.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Spanish Ministry of Science and Innovation for financial support through Project PID2020-116229RB-I00.

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Study of the effect and significance of process variables on the microcellular foaming of different TPUs using supercritical CO_2

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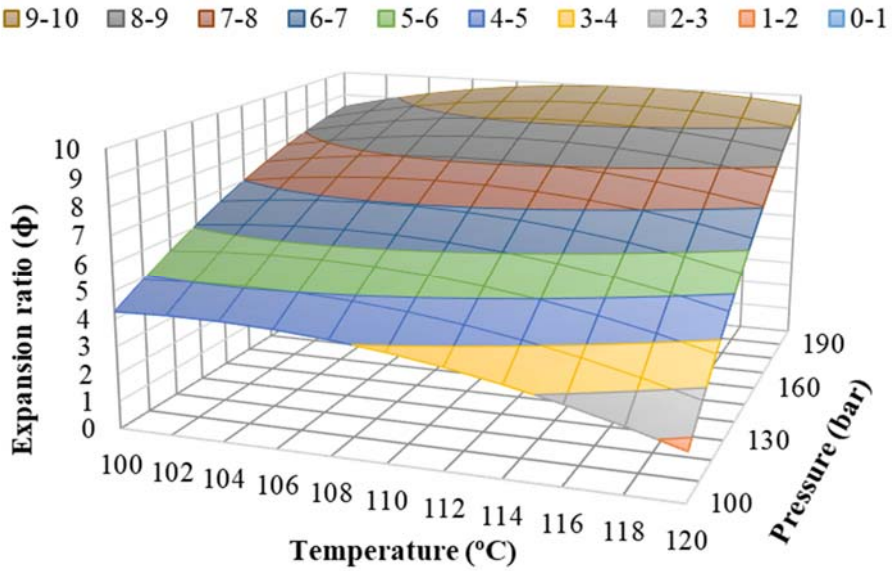
GRAPHICAL ABSTRACT



ABSTRACT

Polyurethanes (PU) are part of the "big six", a group gathering the six most important plastic materials worldwide for their use and economic value. PU is well-known for its outstanding properties such as high resilience, excellent resistance to wear, and long useful lifetime combined with the inherent great tunability of the PU chemistry.

The main problem derivate from the extensive use of these materials is the high number of wastes related to their synthesis and what to do with these materials after their lifetime. For this reason, the present work proposes as an alternative to the traditional recycling of thermoplastic polyurethane the foaming of this material using supercritical CO_2. This new recycling method has some advantages, such as that it is not necessary to reach the melting point of the material. In addition, this alternative provides a new use for the CO_2 that can help it to be integrated in circular economy processes. Finally, should be noted that this alternative not only can provide a new method of PU recycling but also can lay the groundwork of the thermoset polyurethane polymer recycling (which can't be reprocessed by heating).

The present work is focused on the study of the influence and significance of temperature, pressure, and contact time with the supercritical CO_2 in the foaming of thermoplastic polyurethanes (TPU). To carry out the study of these variables, four commercial TPUs were selected. The TPUs selected were 1080A (ester based), 5377A (ester-ether based), 9380A (ether based), and 85085 (aliphatic isocyanate based) from Desmopan catalog of Covestro.

The following range of conditions were studied, using a design of experiments and an statistical software to analyze the data (Statgraphics):

- Temperature: 100 ºC – 120 ºC
- Pressure: 100 bar – 200 bar
- Contact time: 1 h – 3 h

TPUs were characterized using DSC and TGA analysis to determine the melting point and the thermal degradation temperature of the polymer. Once foaming experiments were done, the density of the foams obtained was measured using a 3D scanner SEM analysis were also performed with the aim of analyzing the foam structure obtained. Using the information provided by the density measurement and SEM, cell density (Nf), average cell size (D), and expansion ratio (ϕ) were calculated for the different conditions studied.

The results obtained showed in all cases a high increase of the expansion ratio with the pressure., The temperature leads to an increase of expansion ratio except in the case of the polymer 5377A where this effect is not clear for the range of temperature selected. Contact time did not show a clear influence in any of the cases. Pressure showed an statistically significant influence for all the samples, while temperature effect was only significant for the polymers 9380A and 85085.

The cell density presented a high rise with the increase of the pressure while in the case of the temperature and contact time there was no clear influence on this parameter.

Finally, the average cell size presented a reduction of its value with the increase in the pressure and an increase of its value with the temperature, except in the case of the polymer 5377A where this variable did not present a high influence in the rage of values studied. On the other side, the contact time influence was almost the same for all the samples with the exception of polymer 5377A, where the lowest values of average cell size were obtained after 3 h of contact time. For this property, temperature showed a statistical significant effect for all samples, except for the polymer 5377A, whereas the pressure only was significant in the case of the polymer 9380A and contact time in the case of polymer 5377A.

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Core-shell alginate-chitosan aerogels as drug delivery carriers for oral administration

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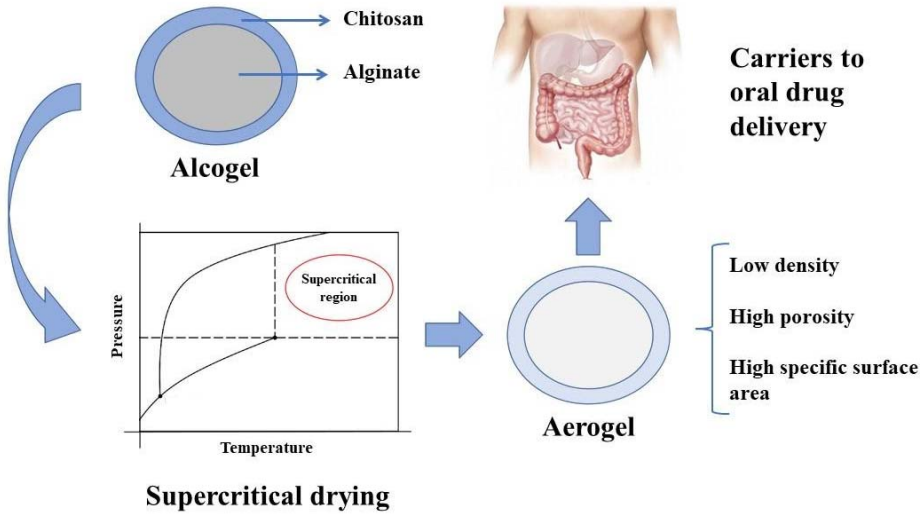
GRAPHICAL ABSTRACT



ABSTRACT

The oral bioavailability and the subsequent efficacy of certain drugs are limited by their low solubility and technological strategies are needed to improve them. Bioaerogels are solid dry materials of extremely low density, high porosity and specific surface area obtained from biocompatible materials for biomedical uses. The properties of bioaerogels make them excellent candidates as carriers for low water-soluble drugs as they can be incorporated in the amorphous form during aerogel processing or upon post-processing. As a result, drug stability and dissolution in biological fluids are facilitated (1). Bio-aerogels can be prepared from hydrogels (e.g. alginate or chitosan) through supercritical CO_2 ($scCO_2$) drying after solvent exchange. $ScCO_2$ drying is able to remove the solvent maintaining the 3D structure.

In this work, combinations of alginate and chitosan are explored to produce aerogel powders and granules for oral drug delivery with different characteristics to modulate the loading and release profile of drugs. Alginate can be easily cross-linked by divalent cations leading to hydrogels of high stability. Chitosan is a mucoadhesive polymer capable of encapsulating different active compounds, modifying their release profiles as a function of pH of the gastrointestinal environment (2).

Alginate gel particles were produced by the ionic gelation method, using calcium chloride as crosslinker. Next, these alginate particles were coated with a chitosan gel layer using

different chitosan sources (molecular weight and/or degree of deacetylation) (3). Finally, the resulting core-shell particles were dried with a continuous flow of $scCO_2$. The resulting core-shell bioaerogels were characterized by N_2 adsorption-desorption, scanning electron microscopy, and helium pycnometry. The thickness of the chitosan shell was determined by optical microscopy. Significant variations in the structures could be observed after the drying process, with high porosity and specific surface areas in all cases. The loading of drugs within these aerogels carriers and the effect of particle design on the drug release profiles will be the subject of further work.

ACKNOWLEDGEMENTS

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Sequential $scCO_2$ drying and sterilization of alginate-gelatin polyelectrolyte complexes (PECs)

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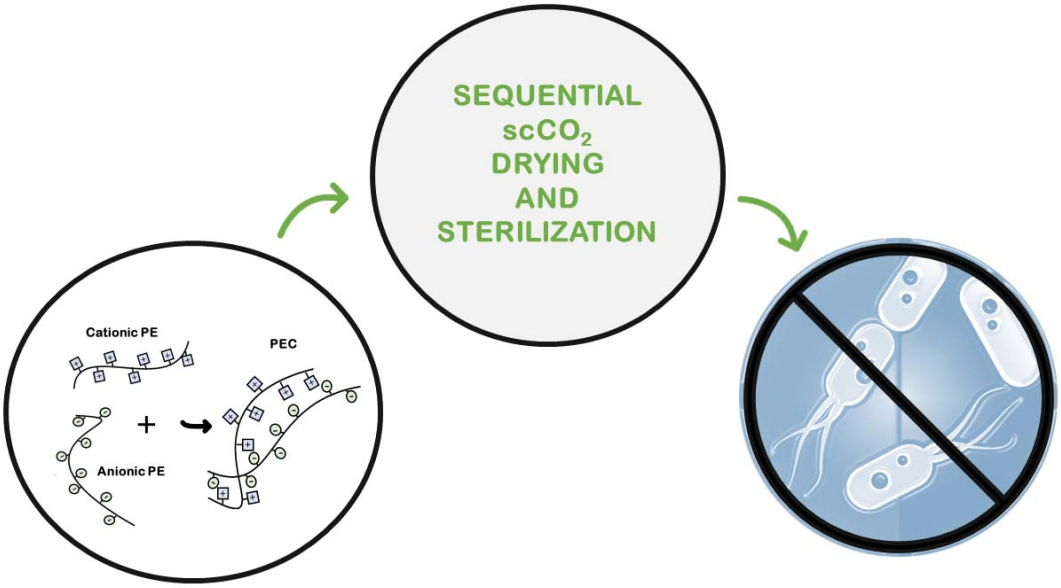
GRAPHICAL ABSTRACT



ABSTRACT

Biopolymers can be used to produce composite scaffolds for tissue engineering, yet the growth of medical applications of such polymers has been decreasing due to the physicochemical degradation occurring during processing/sterilization [1,2]. Standard sterilization procedures compromise the physicochemical and mechanical functional properties of many biopolymers, so the use of supercritical carbon dioxide ($scCO_2$) as a sterilization method appears as a superior and sustainable alternative [1,3]. In this work, a sequential process for preparation and sterilization of gelatin-alginate aerogels using $scCO_2$ was tested. The prepared polyelectrolyte complex (PEC) with alginate and gelatin was then dried and sterilized in a sequential process, using a pouch to simulate the final sterilization step. After drying, two sterilization processing conditions were tested: 100 bar and 250 bar pressure differentials (Δp).

Chemical analysis confirmed the formation of the PEC and also showed that unlike autoclaving (a sterilization comparison method) $scCO_2$ sequential drying and sterilization did not lead to PEC degradation. These results were confirmed by thermal analysis.
Porosity, pore diameter and surface area decreased with increasing Δp during $scCO_2$ sterilization, while the mechanical properties were preserved when the lower Δp was applied. In autoclaved alginate-gelatine PECs the pores collapsed as expected.
Microbiological analyses proved the efficiency of the integrated $scCO_2$ drying and sterilization, as both Δp were able to eliminate natural contaminant microorganisms (e.g., *Bacillus* spp.) from the PECs.
In the end, this innovative strategy made it possible to obtain ready-to-use sterilized PECs with the potential to be applied in regenerative medicine.

ACKNOWLEDGEMENTS
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Supercritical CO_2 drying of non-mammalian gelatin-methacryloyl hydrogels for aerogel formation.

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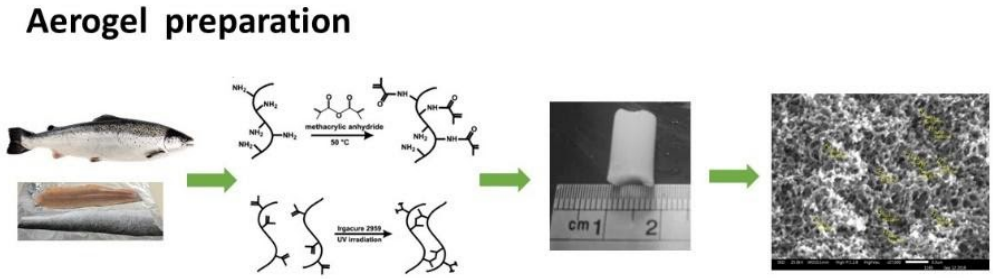
GRAPHICAL ABSTRACT



ABSTRACT

Aerogels have been attracting interest due to their outstanding surface area and defined porous structure relevant for several application such as delivery systems in food and pharma application [1, 2]. Salmon gelatin is a promising low viscosity natural biopolymer that could be used for aerogel formation [3, 4]. Moreover, when gelatin is functionalized with methacrylic anhydride, it can result in a stable hydrogel with fine-tuned mechanical properties and regular microstructure [5, 6]. The objective of this work was to test supercritical CO_2 drying of salmon gelatin-methacryloyl hydrogels for aerogels production as an alternative to traditional drying techniques which often induce shrinkage and collapse of the gel structure [7, 8].

Porous aerogels were obtained from salmon gelatin-methacryloyl hydrogel using a simple and green method based on supercritical carbon dioxide (SC-CO_2) drying. Effects of processing parameters (gelatin concentration, degree of methacrylation and solvent exchange) on the aerogel formation were investigated and optimized for pore size.

Results showed that increasing gelatin concentration and degree of methacrylation improved thermal stability and mechanical properties of the hydrogels. Using a gradient method for solvent exchange with ethanol, porous structure was mostly maintained when supercritical drying was performed at 200 bar and 40°C. The average pore size of the salmon gelatin-methacryloyl aerogel was about 80 nm. The aerogels were stable up to 80 °C. The nanoporous structure produced would show salmon gelatin-methacryloyl

aerogels as promising structuring and delivery systems for bioactives in food and pharmaceutical applications.

ACKNOWLEDGEMENTS
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Chitosan-xanthan gum PEC-based aerogels as innovative solutions for biomedical and environmental applications

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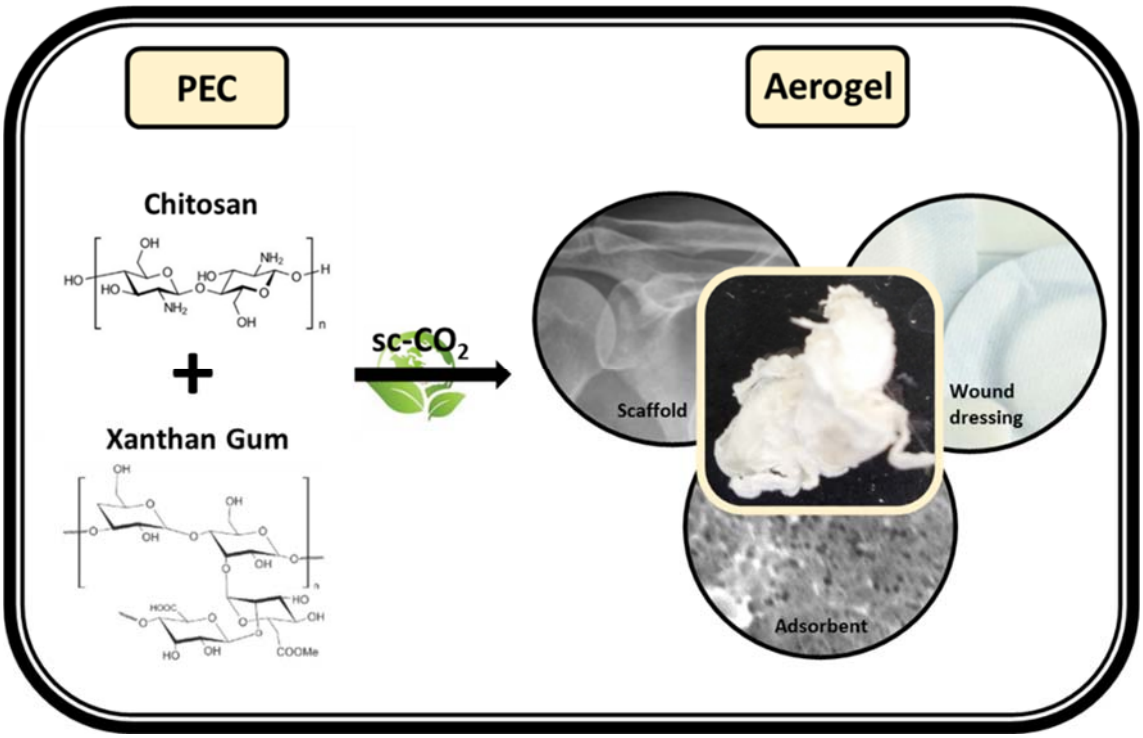
GRAPHICAL ABSTRACT



ABSTRACT

In the scenario of a circular economy and sustainability, the production of aerogels from natural polymeric materials has shown to be quite convenient and to present an enlarged spectrum of potential applications [1,2]. In this work, an innovative aerogel obtained from a chitosan/xanthan gum polyelectrolyte complex (PEC) was developed from the screening of twenty-four different combinations of natural polyelectrolytes: two positively-charged biopolymers (chitosan and gelatin), six negatively-charged biopolymers (pectin, carrageenan, collagen, xanthan gum, alginate and modified galactomannan), and a neutral polymer (guar gum), using the statistical design of experiments (DOE) approach. It has been shown that only the CS-XG PECs yielded aerogels by $scCO_2$-drying at p=150 and p=250 bar and T=35ºC, while all systems provided stable cryogels. The morphology, structure and properties of the CS-XG (1%) materials obtained at two $scCO_2$-drying conditions as well as of the corresponding cryogel were elucidated by various physico-chemical techniques. These materials have potential applications in the environmental and biomedical areas, providing solutions to the current challenges in biomedicine and social, demographic and sanitary life sciences.

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This work was financially supported by Fundação para a Ciência e Tecnologia (FCT), Portugal, through the project STERILAEROGEL – Green method to prepare sterilised biopolymer-based aerogel (POCI-01-0145-FEDER-032625) and Strategic Projects FCT-MEC PEst-C/EQB/UI0102/2019 and UIDB/04539/2020. The authors also acknowledge Professor Marta R. Fontanilla (Universidad Nacional de Colombia) for the supply of the collagen samples.

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Novel modified cellulose-based aerogels for tissue engineering and environmental applications

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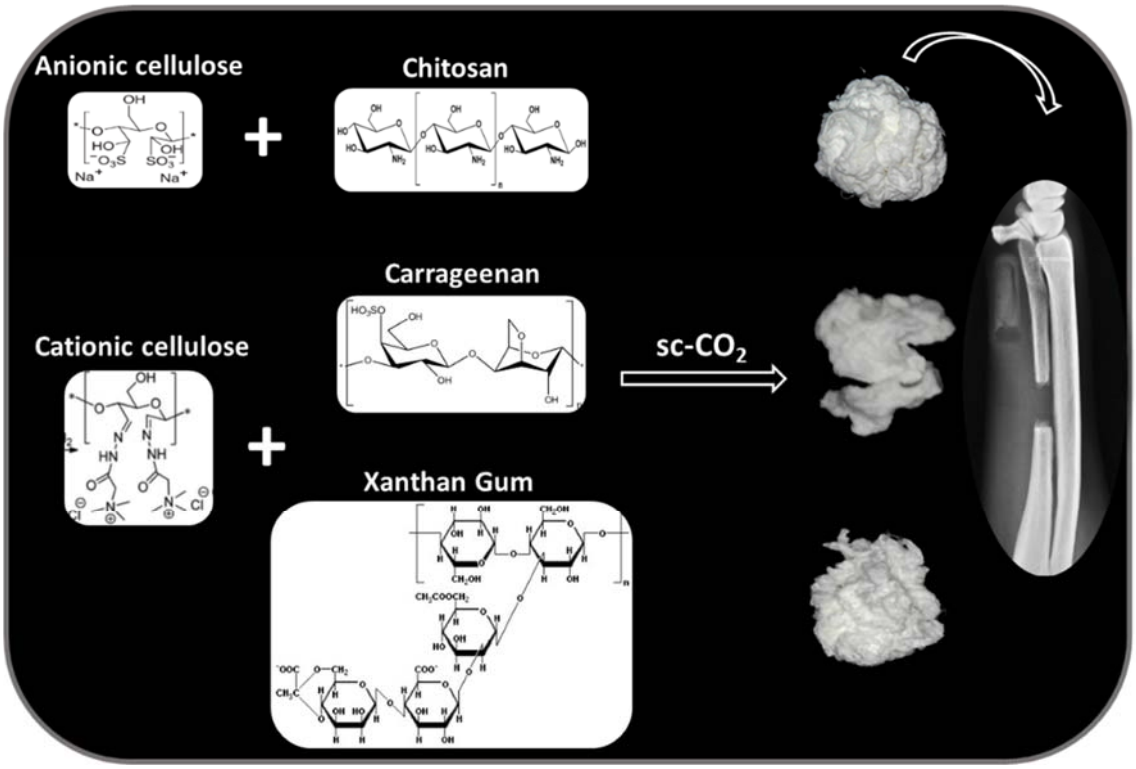
GRAPHICAL ABSTRACT



ABSTRACT

In the past few years, the environmental concerns and economic issues which the global society has been facing, have led to an emergent interest in the exploitation of natural and renewable resources and in green technology to achieve a sustainable development. In this context, biopolymer aerogels have become a very massive accelerating field with very promising applications in health-, food-, pharmaceutical-, insulation-, catalysis- and environmental-related areas [1].

Cellulose is the most abundant biopolymer on earth, owning unique and tunable characteristics, and has been one of the most used polymers in aerogel preparation, either from plant or bacterial sources, with numerous applications in medicine, biosensing, drug delivery, tissue scaffolding and wound dressing [2,3]. Although cellulose aerogels are considered one of the most valuable and environmentally friendly materials, there are still some issues regarding their preparation and properties, particularly as far as natural cellulose materials are concerned. In this work, innovative aerogels obtained from

biopolymers derived from renewable natural resources were produced to be applied in the bone tissue engineering area. Modified celluloses (anionic cellulose and cationic cellulose) were used as the main starting materials for polyelectrolyte complex (PEC) formation, in combination with other ecofriendly, cost-effective and sustainable polyelectrolyte precursors derived from sea residues (chitosan and carrageenan) and microorganisms (xanthan gum). A systematic method involving Design of Experiments (DOE) planning, formation of PEC, FTIR analysis, production of the alcogel, $scCO_2$-drying at p=150 bar and p=250 bar and T=35ºC, or lyophilization was applied. The aerogels and cryogels obtained were characterized by physico-chemical methods and subjected to swelling tests. These materials were suggested for wound healing, controlled drug release and environmental uses.

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Inactivation of *Staphylococcus epidermis* in personalised implants using high-pressure CO_2

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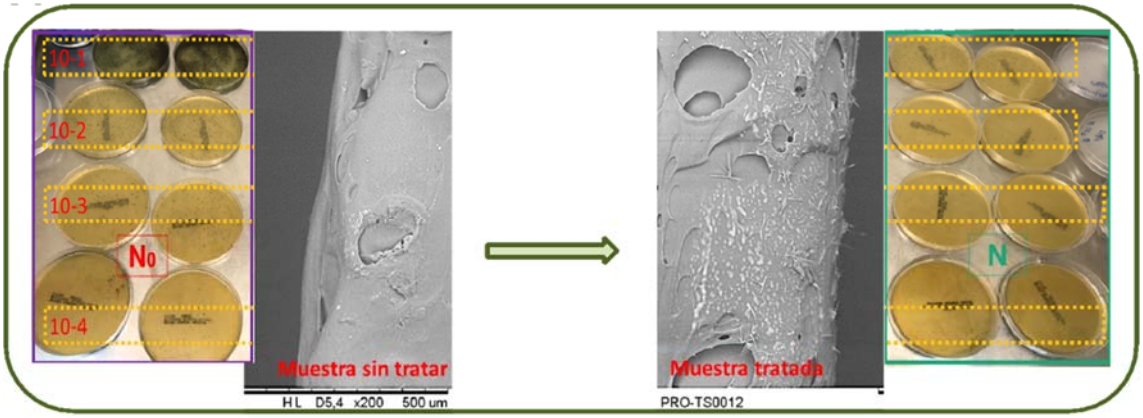
GRAPHICAL ABSTRACT



ABSTRACT

This study explores the effectiveness of inactivating *Staphylococcus epidermis* with supercritical CO_2 (scCO2) on 3D printed polymeric parenteral implants. This fluid was selected because of the relative mildness under the conditions which it operates and its great ability to penetrate solid porous materials.

The investigation was carried out by combining three different sterilization times (15, 30, 60 minutes), depressurization rates (2,5, 4 and 10 bar/min) and additives (isopropanol, acidic water, and hydrogen peroxide) using a Box-Behnken experimental design. The use of additives for the $scCO_2$ made it possible to work without exceeding 40ºC and 100 bar, thus avoiding a greater probability of damage to the implants.

It was possible to achieve > 6 log reductions of the microbial count. The additive was the factor most affecting the degree of inactivation, followed by the depressurization rate and finally the contact time. The best additive was hydrogen peroxide (200 ppm) while the fastest depressurization rate (10 bar/min) of those tested, were the best conditions for carrying out the sterilization process. It was not required more than 15 min of contact time.

Scanning electron microscopy, SEM, showed the effect of the treatment on the surface of the implants, revealing an increase in crystalline microstructures and voids as the severity of the treatment increased. However, in none of the samples was the macrostructure found to be affected. In fact, the presence of micro-cavities at the microstructural level could be beneficial in terms of adhesion of the implant to the prosthesis.

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Particle production

Amorphization Versus Cocrystallization of a Poorly Soluble Drug Using Supercritical CO_2-Assisted Spray Drying

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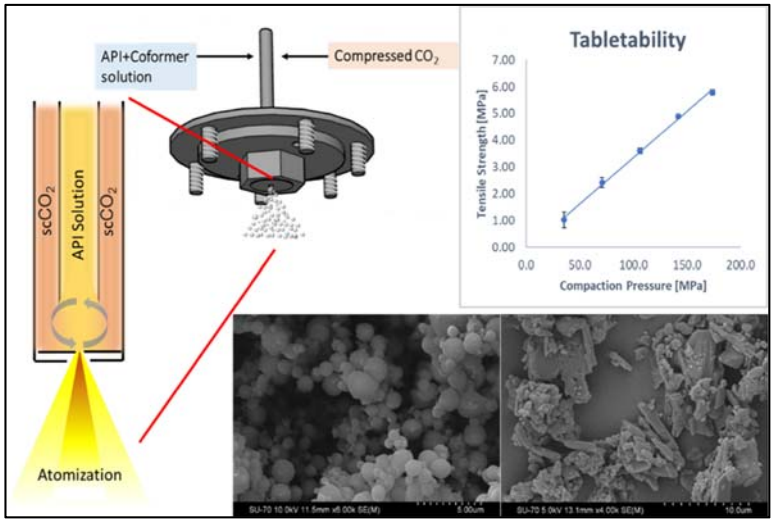

ABSTRACT

The prevalence of poor solubility and thus bioavailability of new chemical entities (NCEs) is a major issue in the pharmaceutical industry, affecting an estimated 90% of drugs in the pipeline [1]. Many methods have been employed to combat this including the formation of salts which is limited to APIs (active pharmaceutical ingredients) with ionizable groups, and amorphization which may cause thermodynamic instability [2]. The formation of cocrystals has proven to be an effective means by which the solubility of an API can be improved, whilst maintaining its stability. The production of micro- and nano-sized pharmaceutical cocrystals by supercritical fluid techniques has proven successful in the past [1]. This study aims to control the particle size and solid state (amorphous vs crystalline) of a model pharmaceutical cocrystal, Celecoxib-Tramadol hydrochloride (CLX-TRA), using the supercritical assisted spray drying (SASD) method.

Feed solutions in methanol and SASD processing conditions were set according to the design of experiments (DoE) in Figure 1, with temperature maintained at 50°C. For the SASD method, an atomization disk with

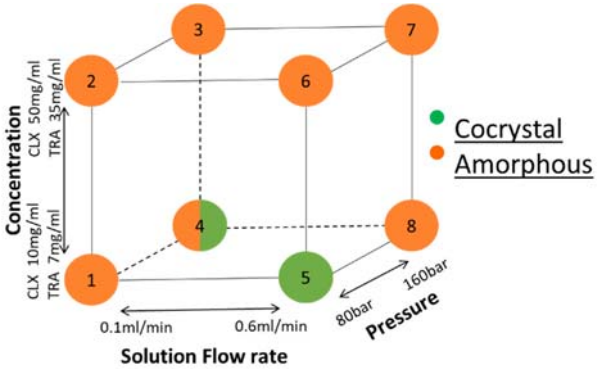

Figure 1. Design of experiment (DoE)

five 40µm holes was placed in the nozzle. Particles were collected on grade 597 Whatman filters.

Powder x-ray diffraction (PXRD) data in Figure 2, highlight the production of primarily amorphous powders for all experimental conditions except those at DoE point 5, which produced the (CLX-TRA) cocrystal under high solution flow rate with low solution concentration and operating pressure. It is presumed that the large solution flow rate and low pressure led to a reduction in the ability to break-up and rapidly dry the solution, whilst low concentrations led to reduction in the rate of precipitation. This is presumed to lead to larger droplets and/or a slower time for solvent evaporation allowing for the production of the stable solid form, the cocrystal. One study observed a similar observation of producing more of a metastable carbamazepine polymorph at higher temperatures (rapid evaporation) [2].

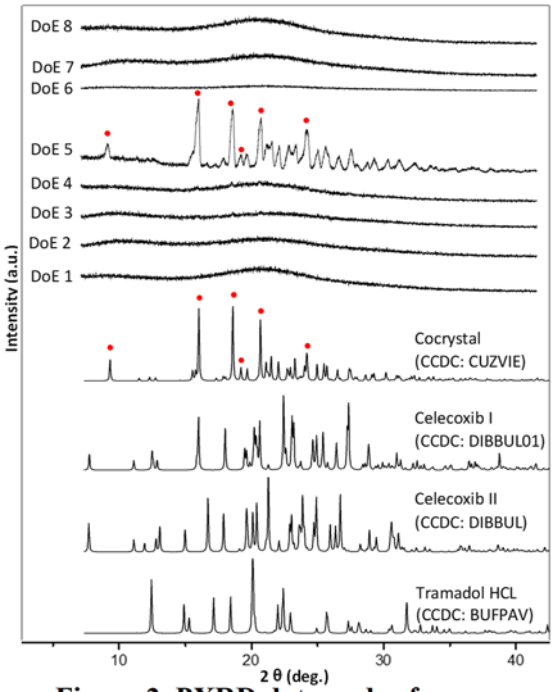

Figure 2. PXRD data and reference

Particle size analysis, determined from scanning electron microscopy (SEM) images, illustrated that increases in solution flow rate and concentration led to larger amorphous particle sizes. Jog et al. observed similar increases in particle size with increasing solution concentration due to a greater solute content in each solvent droplet [3], whilst larger flow rates presumably lead to a larger droplet and possibly droplet agglomeration. Tabletting was carried out using a Gamlen tabletting press to produce 100 mg tablets composed of 10% product (amorphous or (co)crystalline), 5% carboxymethyl cellulose (CMC), 0.1% magnesium stearate and 84.9% microcrystalline cellulose. Thus far, tensile strength data for cocrystalline samples (DoE point 5) demonstrated that at compression pressures at or above 70MPa (2kN), tablets displayed a tensile strength greater than 2 MPa, which indicates appropriate tabletability.

Ongoing work includes determining the effect of particle size and solid-state of on tabletability and dissolution and examining the extent of intermolecular interactions in amorphous samples.

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Formation of novel composites Titanium-Cerium nanoparticles by supercritical antisolvent process

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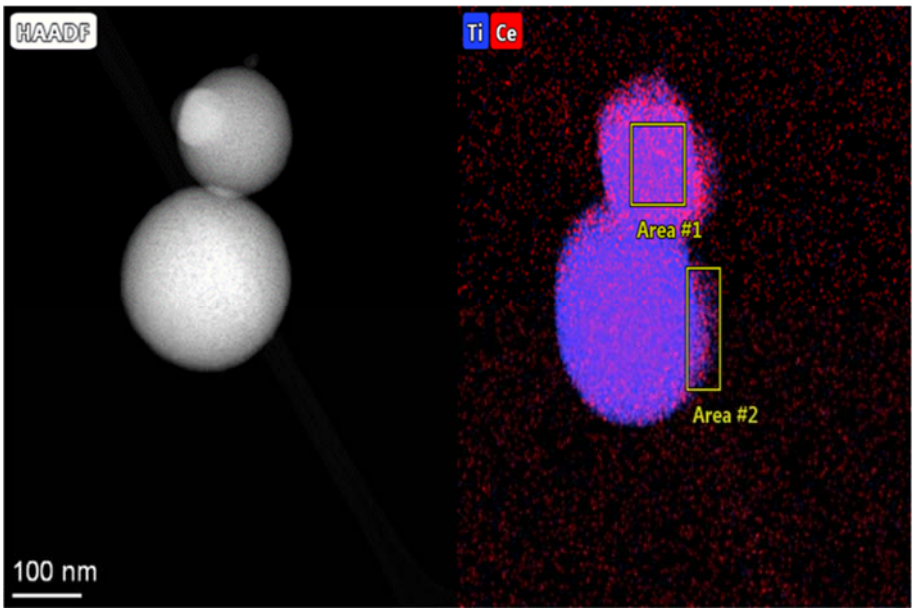

ABSTRACT

Metal oxide nanoparticles are increasingly used in the medical industry for the functionalization of medical implants. This work attempts to deepen into the co-formation of metal nanoparticles by Supercritical antisolvent process (SAS), with the benefits of this process by eliminating the excessive use of organic solvents, thermal degradation or high concentration of solvent. Fractional screening experimental design has been implemented to study the influence of pressure, temperature, percentage of Titanium (Ti) and Cerium (Ce) and concentration. The pressure was set between 10 and 18 MPa, temperature between 313 and 328 K, percentage of Ti 80-50% and Ce 20-50% respectively and finally the concentration was set from 10-20 mg/mL. The SAS plant used corresponds to the SAS 200 model developed by Thar Technology. The SAS process consists of pumping CO_2 into a vessel up to the supercritical conditions at which it is intended to operate. Once these conditions are established, the Ce-Ti solution is pumped into the vessel through a nozzle. The small droplets of the solvent are dissolve in the supercritical CO_2, this causes a supersaturation of the liquid solution, which results in the precipitation of Ti-Ce nanoparticles in powder form inside the vessel walls.

All experiments were carried out successfully in the formation of heterogeneous Ti-Ce nanoparticles. TEM (transmission scanning microscopy) and SEM (scanning electron microscopy) tests show its heterogeneity in size with particles between 40 and 400 nm. Scanning transmission electron microscopy (STEM) shows the tendency in the formation of Ti particles coated with Ce nanoparticles. Physisorption tests show a 3 to 10 times increase in the specific surface area of the combined particles. This makes them viable for functionalization by supercritical impregnation.

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A stability study of cross-linked lysozyme aggregates in supercritical carbon dioxide

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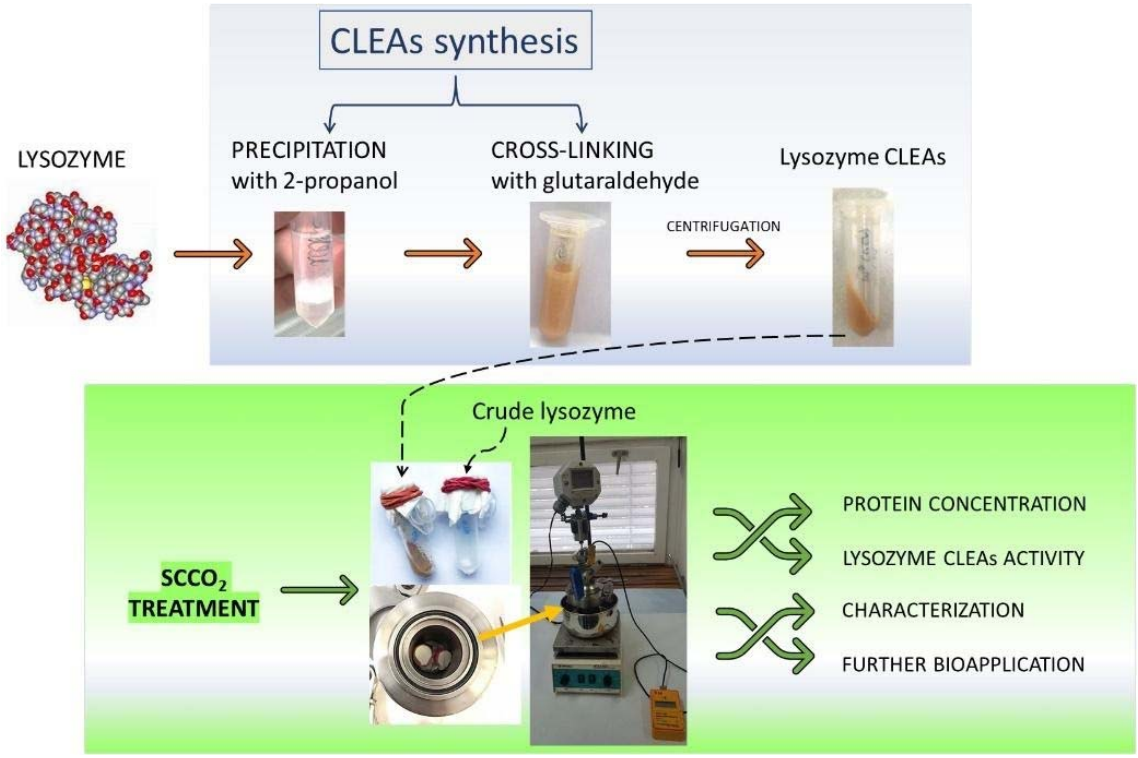

ABSTRACT

Lysozyme is an important bacteriostatic protein, which is widely distributed in nature. It appears likely, that the high efficiency of lysozyme in inhibiting gram-positive bacteria is caused by its ability to cleave the β-(1,4)-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine [1]. In recent years, there has been growing interest in modifying lysozyme via physical or chemical interactions in order to improve its stability in bioapplications. Consequently, immobilization significantly enhances lysozyme's thermal stability and reusability [2]. The technique of cross-linked enzyme aggregates consists of two consecutive steps. The enzyme precipitation occurs in the first step by mixing the enzyme with the precipitant agent (e.g., ammonium sulfate, organic solvents, or polymers) in an aqueous solution [3]. Furthermore, the synthesis of CLEAs continues by cross-linking of the precipitated enzyme into cross-linked enzyme aggregates to obtain

a stable form of immobilized enzyme. Glutaraldehyde (GA) is the most commonly used cross-linker, where enzyme aggregates are irreversibly bonded through covalent bonds between its free amino groups and both aldehyde moieties of GA [4], yielding an insoluble biocatalyst with high stability and activity.

The purpose of the research was to determine the optimal conditions for exposure of crude lysozyme and lysozyme CLEAs in supercritical carbon dioxide ($SCCO_2$) with possible hyperactivation of the enzyme. Therefore, samples of crude lysozyme and lysozyme CLEAs were placed in a thermostated high-pressure batch reactor. The reactor was filled with liquid carbon dioxide (99.5% purity, Messer, Ruše) up to 300 bar. After $SCCO_2$ treatment at different temperatures for a certain time, samples were tested to determine the protein concentration and the activity of an enzyme by the UV-Vis spectrophotometer (Varian Cary Probe 50, Agilent technologies).

The study explores the stability enhancement of lysozyme CLEAs by $SCCO_2$ treatment. It was found that $SCCO_2$ treatment at a pressure of 100 bar had a beneficial effect on the activity of the crude lysozyme and lysozyme CLEAs, as the residual activity under these conditions was the highest in the crude enzyme (211%) and lysozyme CLEAs (190%). With a further increase in pressure, the residual activity decreased. On the other hand, by extending the exposure time in $SCCO_2$, lysozyme CLEAs retain higher activity than crude enzyme, confirming that the immobilized enzyme lysozyme CLEAs is more stable. Optimal conditions for exposure of lysozyme CLEAs to $SCCO_2$ for the purpose of enzyme hyperactivation were achieved at a pressure of 300 bar and 50 ˚C for exposure time of 5 h. The residual lysozyme CLEAs activity under these conditions was 419%, compared to untreated lysozyme CLEAs. The above findings suggest that $SCCO_2$ is a suitable method for increasing the activity of the immobilized enzyme and thus has a positive effect on stability under these conditions.

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