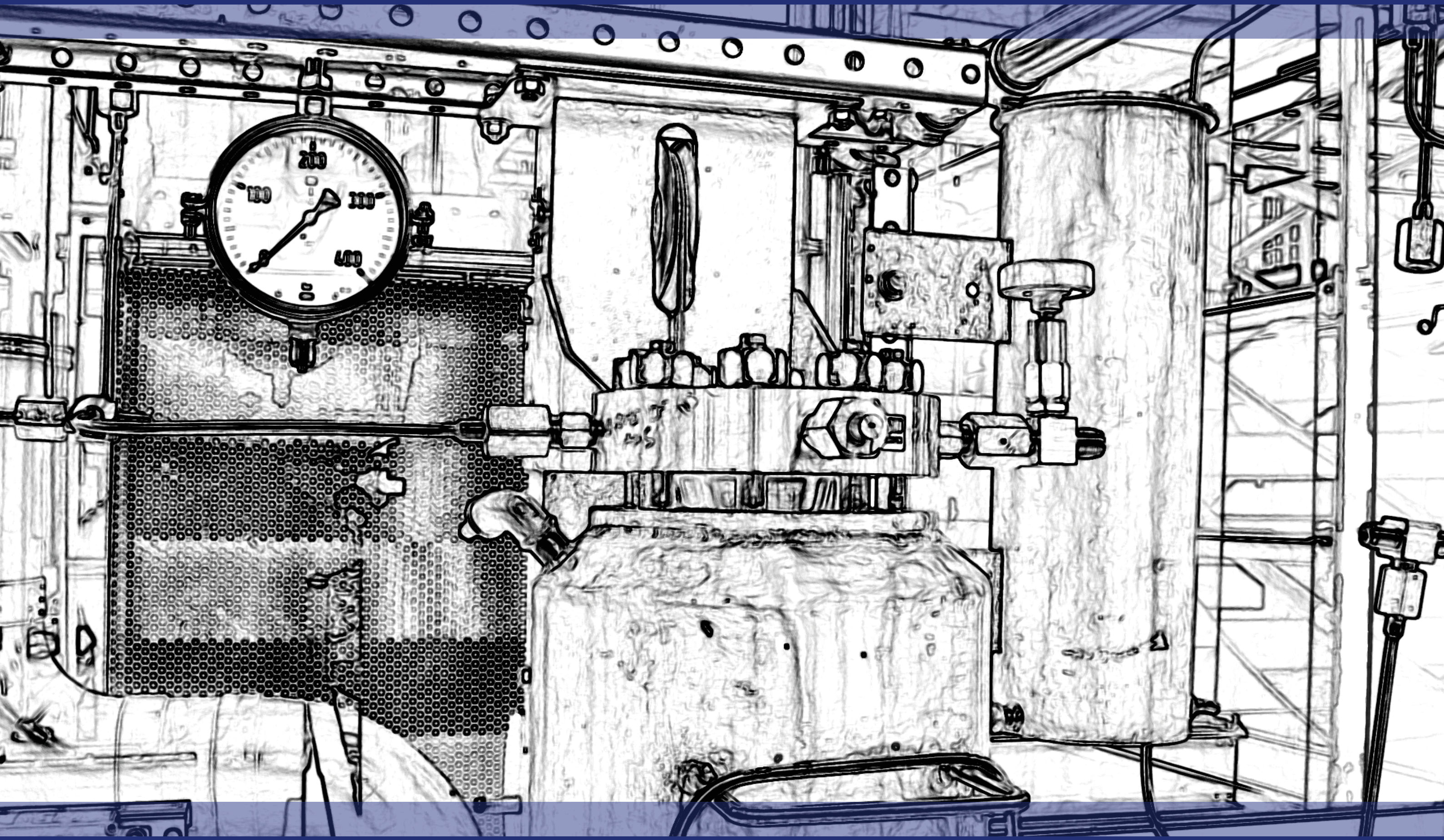
Thomas Gamse, Amra Perva-Uzunalic, Zeljko Knez (eds.)

Book of Abstracts

ESS-HPT 2019



The European Summer School in
High Pressure Technology

07.07. - 21.07.2019

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Preface

The European Summer School in High Pressure Technology (ESS-HPT) is the continuation of many years of high pressure intensive courses. The history of this very successful series of courses started in 1995, when the first intensive course took place in Monselice, Italy. Most of these Intensive Courses were supported by SOCRATES and later Life Long Learning, as shown in following overview:

SOCRATES IP "Current Trends in High Pressure Technology and Chemical Engineering"
- 1995 Monselice / Italy
- 1996 Nancy / France
- 1997 Erlangen / Germany

SOCRATES IP "High Pressure Technology in Process and Chemical Engineering"
- 1999 Abano Terme / Italy
- 2000 Valladolid / Spain
- 2001 Maribor / Slovenia and Graz / Austria

SOCRATES IP "High Pressure Chemical Engineering Processes: Basics and Applications"
- 2002 Graz / Austria and Maribor / Slovenia
- 2003 Budapest / Hungary
- 2004 Barcelona / Spain

SOCRATES IP "Basics, Developments, Research and Industrial Applications in High Pressure Chemical Engineering Processes"
- 2005 Prague / Czech Republic
- 2006 Lisbon / Portugal
- 2007 Albi / France

Life Long Learning IP "SCF- GSCE: Supercritical Fluids – Green Solvents in Chemical Engineering"
- 2008 Thessaloniki / Greece
- 2009 Istanbul / Turkey
- 2010 Budapest / Hungary

EFCE Intensive Course "High Pressure Technology - From Basics to Industrial Applications"
- 2011 Belgrade / Serbia

Life Long Learning IP "PIHPT: Process Intensification by High Pressure Technologies – Actual Strategies for Energy and Resources Conservation"
- 2012 Maribor / Slovenia and Graz / Austria
- 2013 Darmstadt / Germany
- 2014 Glasgow / Great Britain

Unfortunately the financial support for these Intensive Programmes was cancelled within ERASMUS+. The EFCE Working Party "High Pressure Technology" decided in September 2014 to go on with this course in the form of a Summer School.

ESS-HPT "The European Summer School in High Pressure Technology"

ESS-HPT 2015	Maribor / Slovenia and Graz / Austria
ESS-HPT 2016	Maribor / Slovenia and Graz / Austria
ESS-HPT 2017	Maribor / Slovenia and Graz / Austria
ESS-HPT 2018	Maribor / Slovenia and Graz / Austria
ESS-HPT 2019	Maribor / Slovenia and Graz / Austria

The ESS-HPT will take place every year within the first 2 weeks of July at University of Maribor, Slovenia and Graz University of Technology, Austria.

All participants have to give an oral presentation and the abstracts of these presentations, which are peer-reviewed by the EFCE WP Members, are published in this book of abstracts.

The editor

Thomas Gamse
Organiser of ESS-HPT 2019

Many thanks to our sponsors, NATEX Prozesstechnologie GesmbH and Tourismusverband Stadt Graz,

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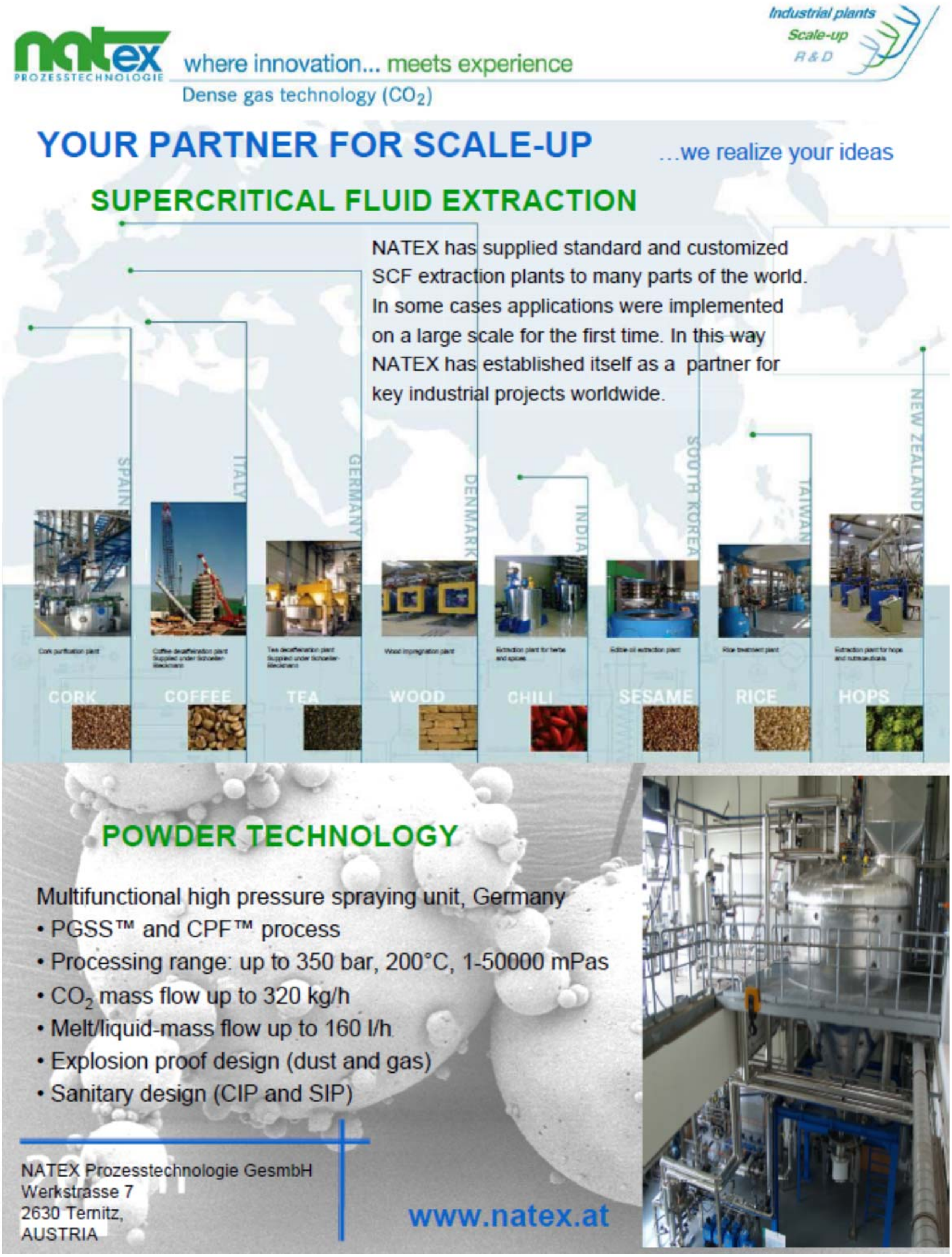

External Additivation of Polymer Parts with high-pressure CO_2 Enhanced Impregnation

Sascha Gilgen

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Introduction

Synthetic polymers as a material have become an integral part of everyday life. Versatile properties combined with high ecological and economic efficiency offer a wide range of applications such as food packaging, building materials, textile fibers, or elastomers. Polymers include additives to meet the required material properties like hardness, UV radiation, aging resistance or coloring. These substances are typically admixed before the original forming process. As a result, the additives are distributed throughout the entire material volume of the polymer part. However, additives such as dyes and UV stabilizers only perform a function in near-surface layers.

The impregnation of polymer parts with compressed CO_2 as a solvent offers an alternative way to apply additives. Additives are embedded in near-surface layers. Hence, additives performing a function only close to the surface are precisely in this position. Depending on the process design, the penetration depth of the additives can be controlled. That results in a possible saving of additives. Besides, it is possible to introduce temperature-sensitive additives, which would not resist the high temperatures during injection molding. [1, 2]

Preliminary experiments carried out so far show very different results depending on the polymer type. Some seem to be easy impregnatable, others hinder the mass transfer of CO_2 and additive or do not resist the depressurization even if it is performed very slowly. This work will contribute to a better understanding of the material transport processes during impregnation. Furthermore, crucial information to design a functioning process is to be obtained.

Experimental

Samples of commercially available thermoplastic polymers are impregnated with real additives or test substances. The effectiveness of the additive in the probe sample is then investigated. By using a well measurable test substance, the mass transferred into the polymer part can be quantified. Due to good results from the preliminary tests, the focus is on thermoplastic polyurethanes. This polymer class is exceptionally versatile. For example, there are polyether and polyester types or aliphatic and aromatic types. Additionally, each chemical composition is also available in various degrees of hardness. It is assumed that these attributes also affect mass transport phenomena.

The samples are treated in a high-pressure view cell equipped with a stirrer. The impregnation process consists of the following steps, as shown in figure 1 in a simplified form. The cell is loaded with a polymer sample and an additive. The selected additive should be soluble in CO_2 or at least dispersible. The cell is then heated up to the desired temperature and filled with CO_2. The experimental parameters are as follows. Temperature between 25 °C and 100 °C, pressure between 6 MPa and 20 MPa, residence time between 5 minutes and 120 minutes. Relief of the pressure terminates the impregnation process. To avoid foaming of the sample, the pressure relief has to be performed slowly with great care. The cell can then be opened and the impregnated sample taken out. Any additive precipitated on the sample is removed before further investigations are carried out.

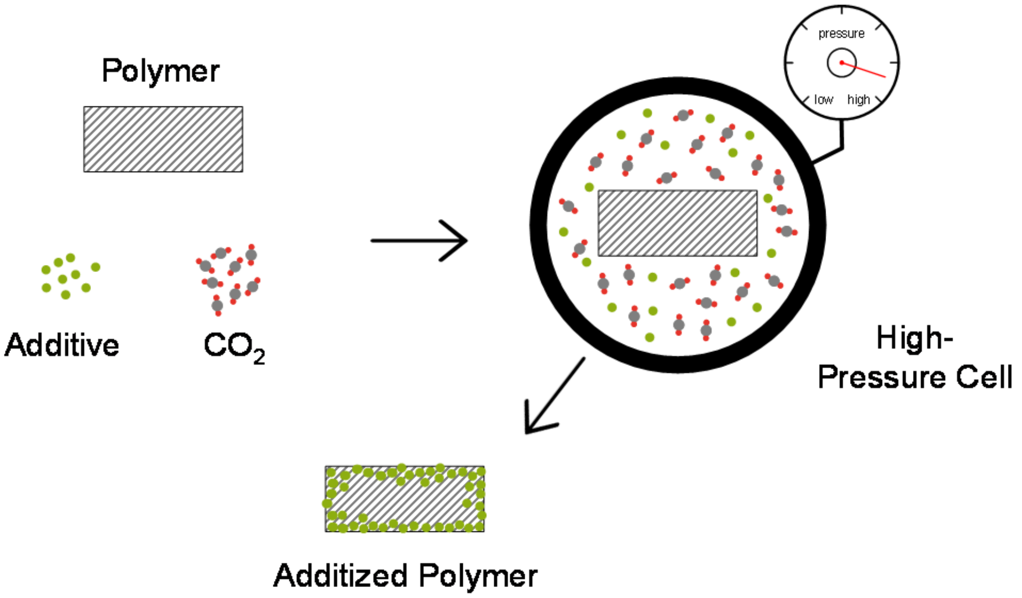

Fig. 1.: Impregnation Steps Simplified

Using a dye as an additive is an excellent way to demonstrate the process and to make an initial estimation of the impregnation capability. The application of a dye also allows the quantitative analysis of the amount of dye contained in the sample. That is the amount of additive that diffuses into the sample and remains there.

This analysis is carried out using UV-VIS spectroscopy. For this purpose, the solid sample must be dissolved in a suitable solvent. In the case of thermoplastic Polyurethane, dimethyl sulfoxide (DMSO) is such a suitable solvent.

A previously dyed sample which is then dissolved in DMSO results in a colored solution which can then be measured in the UV-VIS device. The device illuminates the solution in a cuvette and measures the absorption at different wavelengths from the visible to the UV range. The absorption correlates with the concentration of the dye. To determine the total quantities of the dye contained in the samples, reference solutions of TPU, DMSO, and dye of known concentration are prepared and measured. A similar setup with Polycarbonate as sample material has already been published [3].

In order to determine the mass transport, not only the mass of the sample must be taken into account, but also the surface where diffusion processes take place. It is aimed at spherical samples for easier calculation and to avoid effects at corners and edges.

Summary

Impregnation is a process for the external application of additives. It has the potential to save on additives and makes the use of thermolabile additives possible. CO_2 under high-pressure is used as a solvent. It is non-hazardous and easy to remove by pressure relief. A dye is used as a test substance. The quantity of substance diffusing into a polymer sample is determined. Therefore an impregnated sample is dissolved in DMSO to get a liquid that can be measured in an UV-VIS spectrometer device.

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Comparison of effectiveness of various active substance extraction techniques for wine waste processing

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Introduction

According to the wine laws, wine is an agro-food product obtained by total or partial alcoholic fermentation of grapes suitable for processing to wine. Although it contains a significant percentage of alcohol, wine is not just an ordinary alcoholic beverage. In a world of science, it has been invested enormous effort especially in researching wine chemistry and effects on human health. Wine waste formed during wine production makes up to 20 % (w/w) of total feedstock [1] and fractions of waste products of the most interest are mainly grape pomace and grape cane. Anthocyanins, catechins, phenolic acids and stilbenes are the principal constituents of grape pomace, while grape cane represents more investigated fraction of waste containing high-value phytochemicals with a strong antioxidant capacity. These intermediate products contain biologically active substances, mainly resveratrol and viniferin (Fig.1) which are important in food production and also very useful in a various branches of science including medicine, pharmacy and cosmetic industry [2, 3].

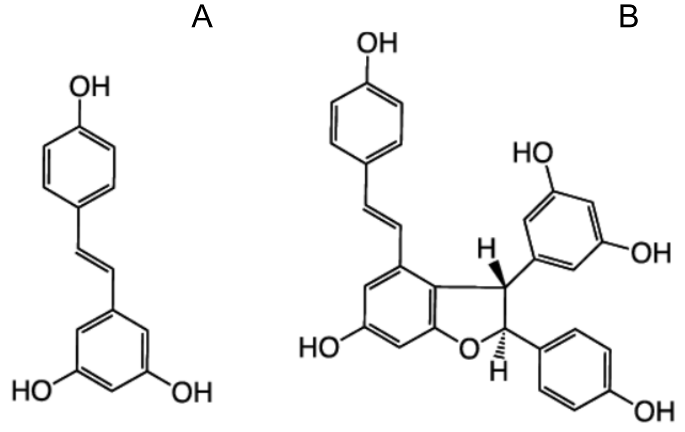

Fig. 1: Structure of stilbenes: A: *trans*-resveratrol, B: *trans*-ε-viniferin

The great interest in these compounds also stems from their biological properties and chemical stability. The mechanism that leads to production of these antimicrobial compounds synthesized and accumulated in different parts of the plant in response to biotic or abiotic stress, is based on self-defense potential of the plants [4, 5]. To obtain a relevant amount of stilbenes for an analytical subsequent processing it is essential to use highly effective extraction methods. In this work various extraction methods for stilbene extraction have been compared. Moreover, a chemical stability of individual compounds under defined conditions was evaluated. Unlike studies conducted to date using aqueous alcoholic solvent, mainly methanol and ethanol [6], for the stilbene extraction, in this study isopropyl alcohol has been utilized as a solvent. The gained extracts have been analyzed by high-performance liquid chromatography in combination with UV/MS detection. This work should be the first step in finding the most efficient extraction methods in general for stilbenes. Future plan will be focused more on high-pressure extraction techniques.

Experimental

Plant Material

- Sample preparation: pruning, drying, chipping, grinding and milling

After pruning, analyzed samples of *Vitis vinifera L.* cv. Cabernet Sauvignon grown in wine region Moravia were dried at room temperature and stripped from seeds while the peels and thimbles were milled. After grinding, material was stored in airtight containers without access to light.

Extraction

- maceration, USE, PLE, Soxhlet extraction

Considering that obtaining bioactive compounds from wine waste require suitable extraction method, several extraction techniques and various conditions were applied and compared.

Separation of Active Substances

- High Performance Liquid Chromatography

The analysis of the obtained extracts was performed by a newly developed HPLC-UV/MS method, which was proved to be accurate, reproducible and efficient for the determination of resveratrol and viniferin.

Detection

- UV/MS detection
- NMR spectroscopy

Using this above mentioned method, three main stilbenes were detected at 306 nm and verification was carried out by MS. Supporting measurements were performed using NMR spectroscopy.

Summary

In earlier work, Soural at al. [7] found that the yield of *trans*-resveratrol from grape cane of *Vitis vinifera* L. cv. Cabernet Moravia was the highest using pressurized liquid extraction with methanol. Another article talking about high pressure techniques for stilbene extraction written by Zachová at al. [8] showed that pressurized liquid extraction was slightly more efficient and much faster than the Soxhlet extraction method. Due to previous results, our work was based on comparison of 4 extraction methods among which one requires higher pressure. In Figure 2 the most significant results are presented. In comparison to other stilbenes, resveratrol was found to be light and thermally stable and the most efficient method proved to be maceration in dark at laboratory temperature, while the most suitable methods for extraction in terms of efficacy targeted to obtain *cis*-ε-viniferin are those avoiding long standing of the extract in solution. Thus, low pressure Soxhlet extraction is the best solution when it comes to obtain the highest yields of *cis*-ε-viniferin. For high yields of *trans*-ε-viniferin ultrasonic extraction carried out at higher temperatures for a shorter time proved to be more efficient than for its *cis*-form.

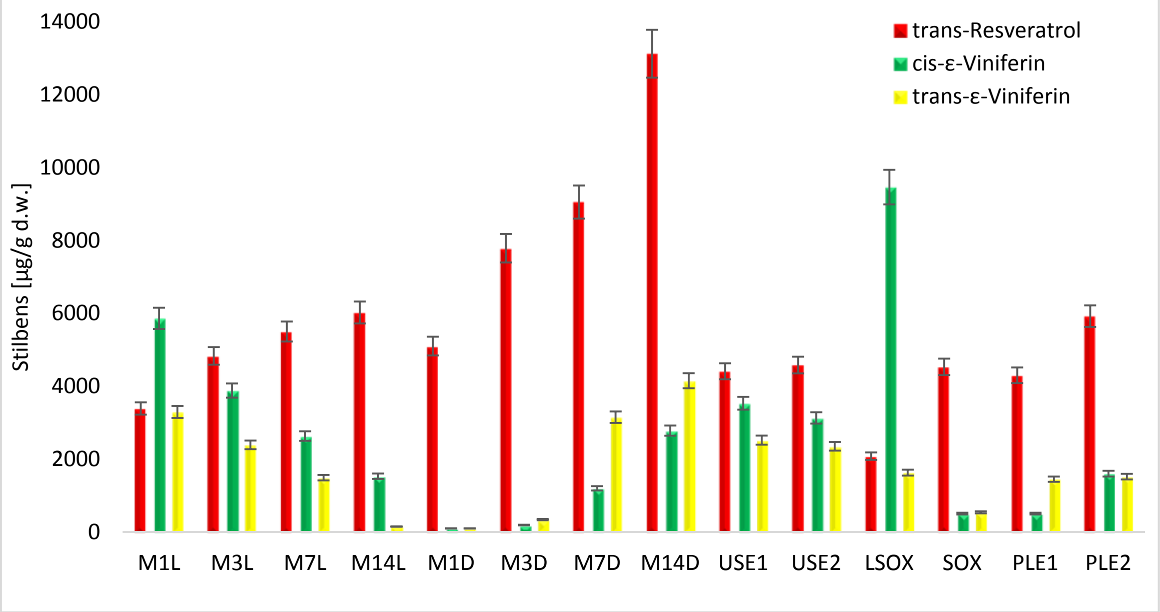

Fig. 2: Concentration of *trans*-resveratrol, *cis*-ε-viniferin and trans-ε-viniferin in µg/g in extract obtained using different extraction procedures (M – maceration at 25 °C, USE - ultrasound extraction at 40 °C, LSOX - low pressure Soxhlet at 40 °C, SOX - Soxhlet extraction at boiling temperature solvent, PLE - pressurized liquid extraction at 100 °C) in the presence of light (L) and in dark (D) in certain time intervals (1-14 days, USE1 – 10 min, USE2 - 20 min, PLE1 - 20 min, PLE2 – 30 min)

Within the stability studies it was found that higher temperature leads to decomposition of stilbenes while light exposure leads to dimerization of *trans*-resveratrol and photoisomerisation of *trans*-ε-viniferin, resulting in observable concentration increase of *cis*-ε-viniferin.

Due to that, the extraction techniques that prevent light exposure of samples are more suitable to obtain resveratrol and *trans*-ε-viniferin. On the contrary, c*is*-ε-viniferin can be more easily obtained after light exposition and also it can be obtained in high quantities from the samples containing other stilbenes upon light exposition. Principal component analysis (PCA) supported using Tukey´s significance test ($p = 0.038$; $p < 0.05$), was used as statistical evidence of a lower velocity of increase in concentration of stilbenes at light (Fig. 3).

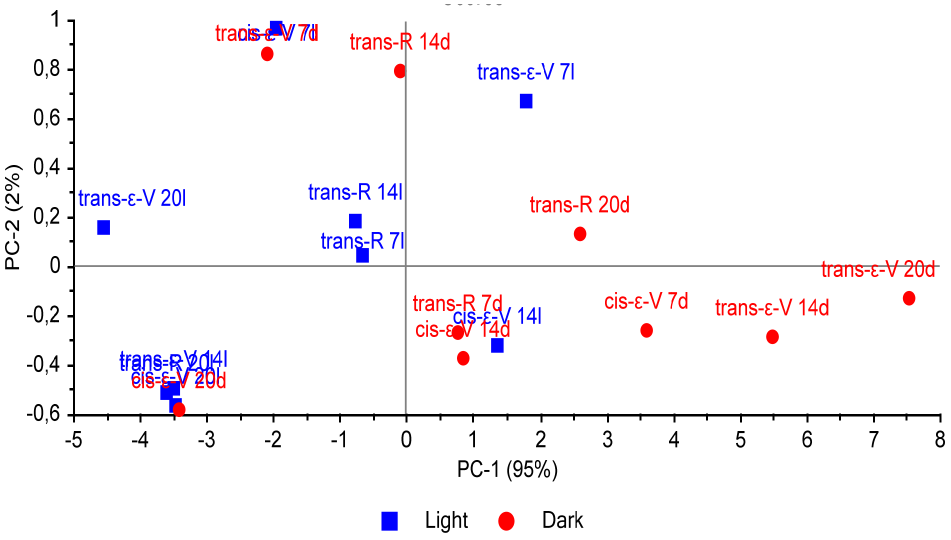

Fig. 3: Principal component analysis showing the statistically significant difference between samples macerated on light and in dark

Acknowledgement

The financial support from the Technological Agency of the Czech Republic via grant no. TJ01000249 is gratefully acknowledged.

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Strategies for the analysis of carotenoids at trace level using supercritical fluids chromatography tandem mass spectrometry (SFC-MS/MS)

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Introduction

Carotenoids are a big class of organic compounds with more than 1100 different known molecules that serve as pigments in multitude of species. These compounds are important in a balanced diet due to their anti-oxidant and photoactive properties, additionally serving as precursors of vitamin A [1]. These compounds are difficult to analyse, as they are easily degraded in the presence of heat, light and oxygen. Furthermore, new methods of analysis with high sensitivity are required in order to discover potential new sources of carotenoids that are also economically viable.

In order to perform qualitative analysis, a chromatographic separation followed by sensitive detection techniques are needed. Commonly, high performance liquid chromatography has been used [2], although the high consumption of organic solvents and the push towards greener and more sustainable chemistry has open the door for other techniques. To this end, supercritical fluid chromatography (SFC) has been gaining ground. Thanks to the versatility of supercritical CO_2 ($scCO_2$), and the possibility to modify its properties with small quantities of organic solvents, a wide range of compounds with different properties can be separated with a selectivity similar or higher to those of more traditional chromatographic techniques [3].

Traditionally, given the highly conjugated isoprene structure of these chemicals, carotenoids have been analysed using photodiode array detection [4]. Nevertheless, this technique has its limitations and is not suited for trace analysis. Mass spectrometry (MS), on the other hand, allows for lower limits of detection and opens the door for quantitative analysis of trace concentrations of carotenoids. Electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) have been found suitable for the analysis of carotenoids [2].

In the present study, the development of a new methodology for the quantitative analysis of trace concentrations of carotenoids is presented.

Experimental

Thirteen carotenoids of commercial importance, shown in Table 1, were selected for the method development.

The analysis of these carotenoids was performed using an Infinity II SFC chromatograph coupled to a 9495 Triple Quad LC/MS mass spectrometer. For the separation, a 1-aminoanthracene chromatographic column was used. This column has been reported as being suitable for the analysis of carotenoids in a prior study [3]. $ScCO_2$ was used as mobile phase, with methanol acting as a co-solvent.

Multiple reaction monitoring was selected for mass spectrometry as it offers the best sensitivity for the quantification of chemicals. Identification was performed by the selection of two unique transitions for each compound. Quantification was done by means of external calibration and relative response to an internal standard. Two ion sources were compared (ESI and APCI) in order to decide which one performed the best in terms of ionisation efficiency and response.

The optimisation of both the chromatography and the ion source was performed by means of design of experiment.

For the validation of the method, extracts from the copepod species *Eudiaptomus gracilis* were analysed. Solid-liquid extraction and supercritical fluid extraction were applied to obtain the carotenoids from the copepods.

Summary

Carotenoids have been successfully separated using the new SFC method (Fig. 1). Different proportions of $scCO_2$ and MeOH can greatly affect the separation of structurally similar carotenoids, as well as different conditions of flow, pressure, and temperature. Regarding ionisation, both ESI and APCI seem to offer similar results, obtaining similar limits of detection in the range 0.1 – 6.2 ng ml^{-1}.

The method was validated with the extracts from *E. gracilis*. Trace concentrations of most of the analysed carotenoids were found. A detailed description of the results will be offered during the presentation.

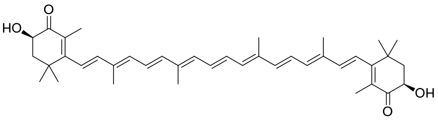
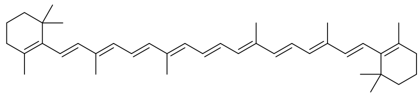
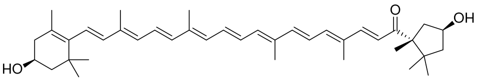
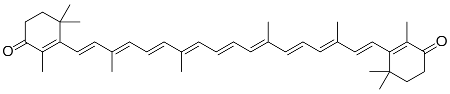
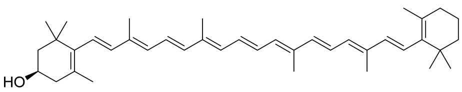
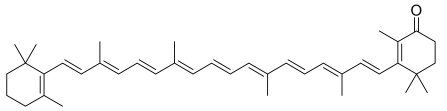
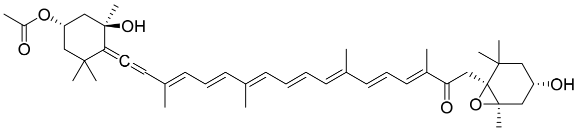
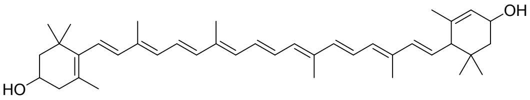
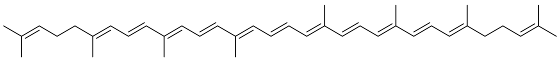
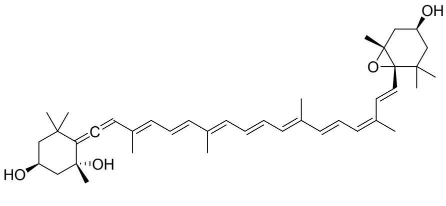
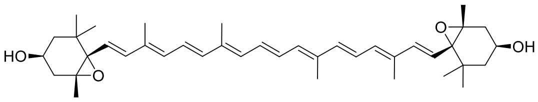
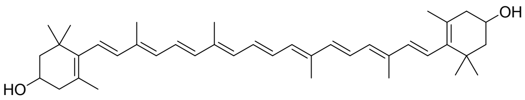
Name	#CAS	Structure
Astaxanthin	472-61-7	
β-carotene	7235-40-7	
Capsanthin	465-42-9	
Canthaxanthin	514-78-3	
Cryptoxanthin	472-70-8	
Echinenone	80348-65-8	
Fucoxanthin	3351-86-8	
Lutein	127-40-2	
Lycopene	502-65-8	
Neoxanthin	30743-41-0	
Violaxanthin	126-29-4	
Zeaxanthin	144-68-3	

Table 1.: Name, CAS number and structure of the selected carotenoids

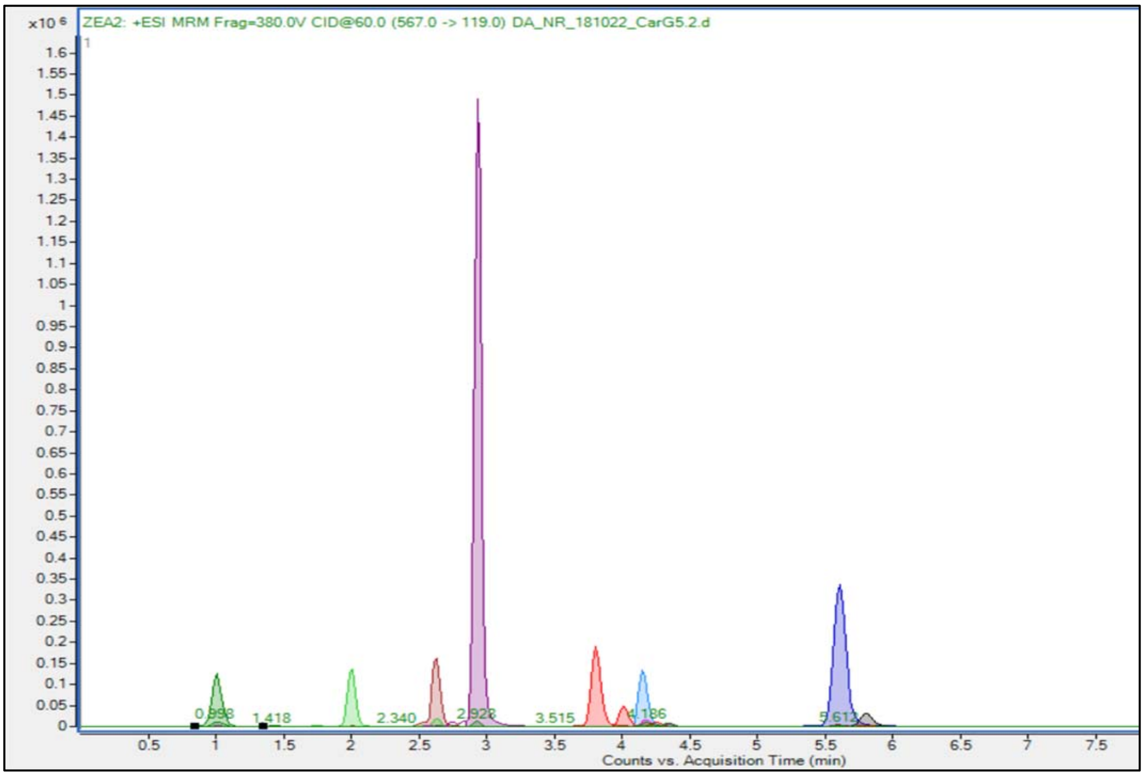


Fig. 1.: Reconstructed chromatogram at a concentration of 50 ng ml^{-1} of the target carotenoids.

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Modeling the Industrial High-Pressure Polymerization Process of EVA-Copolymers

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Introduction

Since the dawn of humanity, societies rely on resilient materials meeting the necessary physical demand to withstand permanent application. With the engineering ingenuity of the 20th century came the discovery of a new type of material: synthetic polymers. Due to their high versatility, lightweight, ease of processing and mass-production, high resilience as well as moldability to complex architectures, polymers have now outgrown most human-made materials with a production of 322 million tons in 2015.[1,2] An example for a widely applicable polymer is polyethylene-vinyl acetate (EVA), a copolymer containing ethylene and vinyl acetate. EVA-copolymers contain a diverse spectrum of materials with a broad range in applications. Next to others, EVA-copolymers are employed in the wire and cable industry for heat shrinkable insulation, semi-conductive insulation jackets and flame retardant insulation.[3]

During the last decades, process modeling generally advanced to a substantial element of process design and optimization. This is due to its potential to substitute time- and cost-intensive experiments.[4] Modeling approaches of industrial polymerization processes of homopolymers such as polyethylene are well-known in literature.[5] However, published approaches of modeling industrial EVA-copolymer processes are scarce. Therefore, a new deterministic model of the radical copolymerization of ethylene and vinyl acetate in an industrial multi-zone autoclave reactor with the software Predici by CiT has been developed.

Modeling

The developed deterministic model of the radical copolymerization of ethylene and vinyl acetate is based on the kinetic network of free radical (co-)polymerization. This includes initiation, propagation, termination as well as several transfer reactions. In addition, a

CTA (chain transfer agent, modifier) and its transfer kinetics are implemented in the model.

Apart from kinetics, the model includes the reactor geometry. Hereby, an industrial multi-zone autoclave is depicted as a series of CSTRs (continuously stirred tank reactors). This is due to the assumption, that back mixing between zones is negligible.[6]

In addition to the established reactor geometry, a numeric iterative recycle stream is included in the model. Hereby, the recycle stream connects the autoclaves exit- and feed streams. Including a recycle stream is crucial in order to improve the depiction accuracy of the actual EVA-polymerization process: by including a recycle stream, the accumulation of substances in the plant can be investigated and their influence on the average molecular weights of EVA-copolymers can be determined. For the implementation of the recycle stream, an iterative method was employed. By means of this, the recycle values are set zero for the first iteration, while for every consecutive iteration the low-molecular output of the reactor in the previous iteration is set as recycle stream. This iterative loop is continued until the recycle stream values (e.g. mass flow, composition) converge and stay constant. To consider an additional recycle related aspect of the industrial process of EVA-copolymerization, a purge stream is added to the model as well. With this purge stream, the modeled multi-zone autoclave’s exit stream is reduced by a certain percentage of the fresh feed (*purge value*) before being set as the recycle stream for the next iteration. With this, the relation of accumulation and purging is to be investigated.

In figure 1, the modeled recycle stream development over a course of 250 iterations is shown for different constant purge values. Hereby, the fresh feed consists of fixed values (mass flow and composition), while the recycle stream is updated with each iteration.

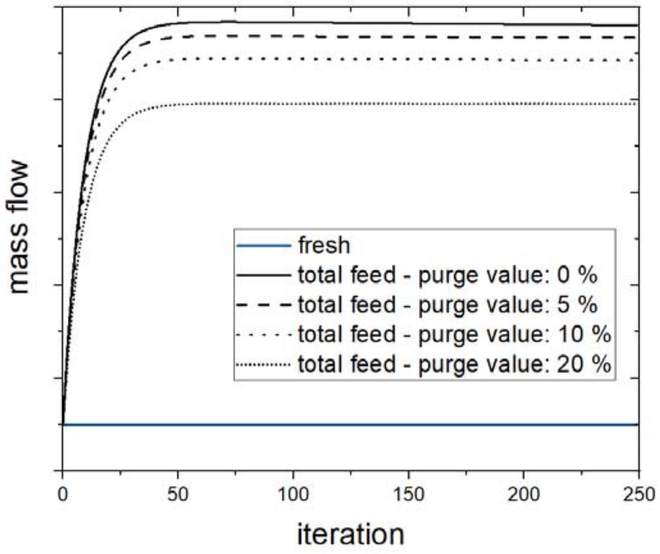

Figure 1: Modeled total feed stream development of mass flows as a function of purge values. Hereby, the total feed stream consists of the sum of fresh and recycled feed.

As shown in figure 1, the mass flow of the recycle stream increases for a number of iterations, until the modeled system converges. Hereby, the magnitude of final mass flows is influenced by the employed purge values. For higher purging values, where a higher percentage of the recycle stream is removed, the overall feed masses are lower. In order to investigate the composition development of the recycle stream, the iteration dependent mass fractions of ethylene, vinyl acetate and the CTA is shown in figure 2.

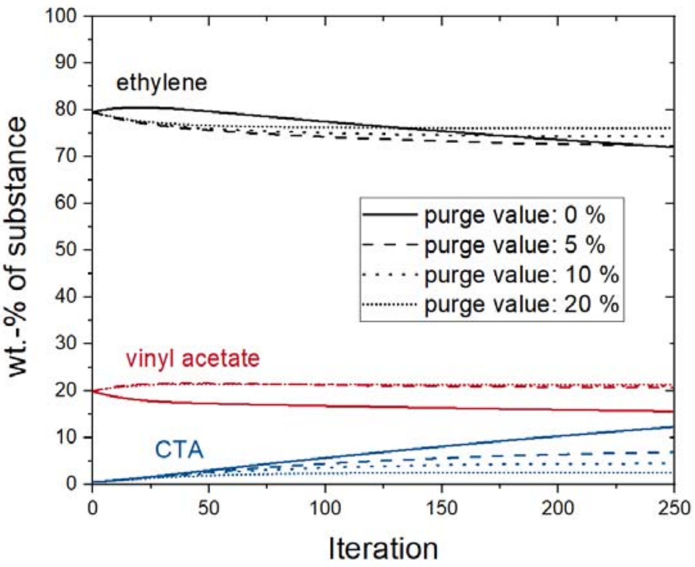

Figure 2: Modeled total feed development of composition (mass fraction) as a function of purge values. Hereby, the total feed stream consists of the sum of fresh and recycled feed.

In terms of composition development (see figure 2), accumulation of modifier and depletion of monomer species within the system is observed without purging. This is a result of the implemented kinetics. While both monomer species are roughly consumed at the same rate, since the copolymerization is close to ideal at process conditions, this is not the case for modifier. With a separate set of kinetics, the treated modifiers conversion is only a fraction of the monomer conversion. This difference in consumption results in accumulation of the less reactive species, which in case of the treated copolymerization is the modifier. In regard to the influence of purging, figure 2 shows that with higher purging values the accumulation of CTA is reduced.

Resulting from different levels of CTA accumulation, an influence on the average molecular weights of modeled polymers is observable in dependence of purging values. This relation is shown in figure 3. Hereby, average molecular weights are decreasing with increasing purge values. This is due to the fact, that with increasing purge values the accumulation of CTA in the system is significantly reduced. As a result, chain transfer reactions are less frequent and chain lengths (average molecular weights) are larger.

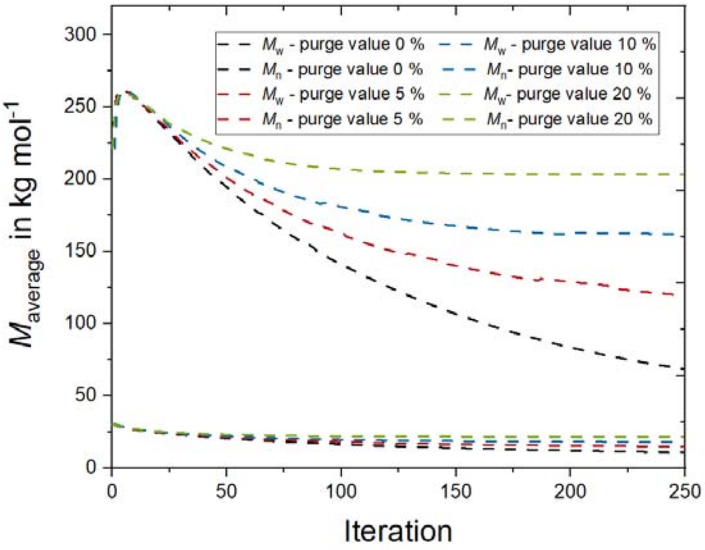

Figure 3: Modeled average molecular weight development as a result of CTA accumulation.

Summary

A new deterministic model of the copolymerization of ethylene and vinyl acetate in industrial multi-zone autoclave reactors was developed. Hereby, the model includes a numeric recycle stream. This is crucial in order to improve the depiction accuracy of the actual EVA-polymerization process: by including a recycle stream, the accumulation of substances in the plant can be investigated and their influence on the average molecular weights of EVA-copolymers can be determined.

By simulation of various settings, it was found that purging of the recycle stream directly influences the magnitude of CTA accumulation, which consequently influences the average molecular weights of simulated polymers.

The next step for the set up model is the validation with experimental data. With this, the overall concept of modeling recycled CTA accumulation to determine influences on average molecular weights may be verified.

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Hydrothermal pretreatment of lignocellulosic biomass and valorization of hemicellulose hydrolysates

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Introduction

Today energy, fuels as well as a large variety of chemical products are derived from fossil resources [1]. However, as non-renewable resources their future availability is inherently limited [2]. While energy can be derived from various renewable sources such as solar or wind power, fuels and especially chemical products require materials that need to be either completely synthetized, recycled or derived from regenerative feedstock in the future [3].

A possible way to ensure the long-term supply of necessary materials for modern chemical products is the use of biomass as a substitute resource for hydrocarbons, which are traditionally derived from fossil resources [4]. Furthermore, this offers the opportunity to acquire complex naturally synthesized substances for the production of novel bio-based products [5]. The corresponding process concepts are widely referred to as biorefineries, in analogy to petrochemical refineries [3].

First generation biorefineries use relatively easily processible biomasses, such as food crops or dedicated oil crops to produce mainly fuels [3]. However, the resulting competition with food supply and arable land-use raised ethical concerns [6]. Therefore, for the second generation of biorefineries the utilization of feedstock from non-food and abundant biomasses such as lignocellulosic residue streams from forestry- and agricultural industries is pursued [6,7].

Lignocellulosic biomass is found mainly in terrestrial plants. It is composed of the natural biopolymers cellulose, hemicellulose and lignin, as well as further components in smaller quantities (e.g. protein, fats, ash) [8]. The overall biomass composition strongly depends on the plant species, used plant parts, as well as growth- and in general also storage conditions [9,10]. Additionally, the availability of certain biomasses as raw material may change periodically as caused by growth- and harvest cycles, as well as irregularly due to market dynamics [11]. Thus, robust and flexible processes, able to handle biomasses from different sources and of varying quality are desirable.

Due to its complex structure and its recalcitrance lignocellulosic biomass is generally not directly usable as a feedstock for (bio-)chemical production processes [12]. Pretreatment steps are necessary to separate the different fractions and render them accessible for further conversion or purification steps in order to produce value-added products. These pretreatment processes typically have an essential impact on the overall process cost and the achievable product quality [13].

Past economic studies suggest that multiple different fractions of the biomass should be converted to high value products in order to ensure an economically feasible and competitive process [14–17]. However, current lignocellulose pretreatment concepts suitable for full fractionation and the production of chemicals instead of fuels from biomass are often still expensive and energy intensive and thus hardly suitable for sustainable realization in production scale. Typical challenges are the efficient solubilization and fractionation of the recalcitrant biopolymers and the selective break-down into their components [3]. Due to the resulting complex mixtures of several different compounds, which depolymerize, react, degrade and in some cases repolymerize it is challenging to achieve reasonable yields and qualities for each target fraction [18–20].

A variety of different pretreatment methods for lignocellulosic biomass have been proposed of which not all are suitable for the valorization of multiple biomass fractions. They reach from physical, chemical, physicochemical to biological processes and have varying levels of maturity from lab scale to first production plants [13]. Due to the strong interplay between biomass species, pretreatment method and target products, it is unlikely that one singular method will be applicable for all purposes [13].

In Germany, wheat straw is one of the best available lignocellulosic biomasses [21]. A promising approach for a full fractionation of wheat straw is the liquid hot water (LHW) process (physicochemical pretreatment). During LHW the biomass is treated with sub-critical water at temperatures of typically 170-230 °C and pressures of up to 50 bar [13]. The solid hemicellulose is depolymerized and solubilized, releasing pentose and hexose monomers/oligomers, acetic acid and degradation products into the aqueous fluid phase. This hydrolysate can be collected as the hemicellulose fraction. Lignin dissolves only partially (up to 20 %) at these process temperatures [22]. Most of the lignin and the cellulose are largely only structurally altered [23,24] but not dissolved [25–27]. In subsequent process steps the cellulose in the solid residue can be enzymatically hydrolyzed to yield a glucose solution and solid lignin [28].

Outlook

At the Institute for Thermal Separation Processes at the TUHH a hydrolytic pretreatment process with only liquid hot water flowing through a fixed bed of straw has been established and experimentally validated at a scale of 300 t/a of pretreated biomass [32]. However, the further up-scaling potential of this reactor concept is currently limited, due to mechanical stability issues of the compressible fixed-bed [33]. To tackle these issues the process and/or reactor design have to be improved.

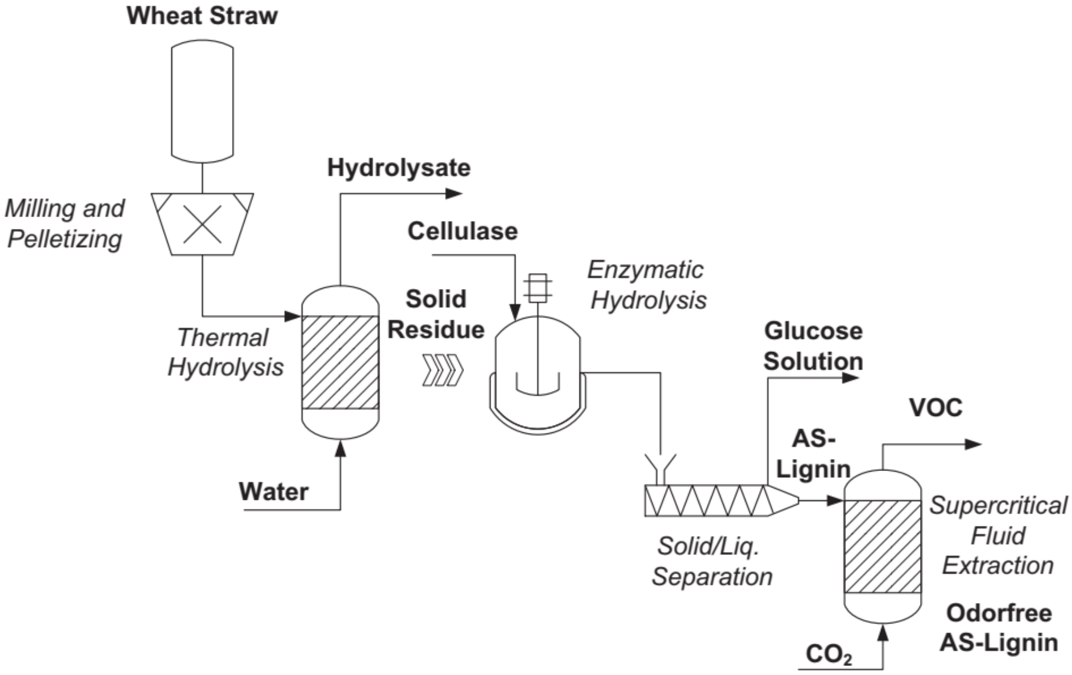

Fig. 1.: Scheme of the LHW process at the TUHH for the fractionation of wheat straw [34]

Further, understanding the biomass solubilization mechanisms is important for ensuring high selectivities and process control, avoiding unwanted product degradation or targeting specific degradation routes and allowing the design of scalable processes for the flexible and robust production of multiple high value products from different lignocellulosic biomasses. These pretreatment processes should optimally be designed together with concepts for suitable down-stream processes. Typically, the hemicellulose fraction in hydrothermal lignocellulose biorefineries is obtained as a dilute aqueous mixture of several highly reactive and thermally sensitive carbohydrates. Thus many classical thermal separation processes are unsuitable for the concentration and purification of its components [35] which would allow its valorization. The design of novel purification methods for aqueous hemicellulose hydrolysates would benefit the discussed lignocellulose biorefinery concept, to some extent even independently of the applied hydrothermal reactor/operation concept.

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Recovery of astaxanthin pigments from marine crustacean waste streams using supercritical fluid technology

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Introduction

Food processing industries produce large quantities of by-products.[1] Expansion of the crustacean processing industry has been accompanied by large amounts of waste. Annually, around 6 million to 8 million tonnes of marine crustacean waste are produced worldwide representing an opportunity to convert them in valuable resources.[2] Crab shell waste represent a valuable raw material for different applications as they contain large amounts of chitin and carotenoids pigments.[3] In this field, brown crab (*Cancer pagurus*) wastes have been identified to be a promising source of astaxanthin pigments.

Astaxanthin (ASX) is the most abundant carotenoid found in marine animals such as crab, lobster and shrimps including their processing waste.[4] This reddish-pink pigment is derived through oxidative transformations of ingested β-carotene or zeaxanthin from feed microalgae.[5] It is also reported that astaxanthin has several biological activities, including antioxidant, anti-lipid peroxidation, anti-inflammation, anti-diabetic, cardiovascular disease prevention, anticancer and immuno-modulation.[6]

Astaxanthin from crab wastes are mainly extracted through traditional solid-liquid extractions using organic solvents.[3,7–10] These methods have several drawbacks, such as long extraction time, evaporation of a huge amount of solvent, stability problems, batch-to-batch variations, low selectivity and relative low yields.[5,11] Therefore, it is a key focus to explore greener extraction methods with faster extraction rates and higher astaxanthin extraction yields.

The use of supercritical fluids is an alternative extraction technique that can help to overcome these limitations. This technique uses GRAS solvents, reduces the time of extraction and are ideal for the extraction of thermolabile compounds such as astaxanthin.[5,12] The most commonly used supercritical fluid is CO_2 which has low viscosity, low surface tension, high diffusivity, non-toxic, non-flammable, inexpensive, widely available, chemically inert under and recyclable.[4,12] Furthermore, the absence of oxygen in the extraction system is other advantage of this methodology because the presence of oxygen is one of the factors that affect astaxanthin stability.[4,11] The limitation of supercritical CO_2 as solvent is that it is not suitable to extract polar compounds. However, the addition of a modifier, such as ethanol, can significantly improve extraction efficiency.[4]

Therefore, the purpose of this study was to exploit the use of supercritical carbon dioxide extraction with ethanol as co-solvent to obtain an astaxanthin-rich extract form brown crab waste. A full factorial design and response surface methodology were applied to determine the influence of extraction process conditions, namely equilibration time, pressure, temperature and extraction flow rate on the total extraction yield, astaxanthin extraction yield and astaxanthin concentration in extracts.

Experimental

Chemicals. The chemicals used for different extraction methodologies were: carbon dioxide 99.95 % from Air Liquide (Lisbon, Portugal), ethanol absolute 99.9 % from Carlo Herba (Val de Reuil, France). For phytochemical characterization, astaxanthin ≥97 % and dimethyl sulfoxide 99.9 % was purchased from Sigma-Aldrich (St Quentin Fallavier, France).

Raw material. Brown crab (Cancer pagurus) residues were kindly provided by Tejo Ribeirinho, Lda., Portugal. The raw material weas dehydrated in a Coolsafe Superior Touch 55-80 freeze dryer (Scanvac) at -55 ºC. After freeze-drying, the residues were milled using a cutter-emulsifier CKE-8 (Sammic, Azkoitia, Spain) and the particle size of ground material was determined using an AS 200 basic vertical vibratory sieve shaker (Retsch, Haan, Germany), with a measuring range between 250 µm and 710 µm. The processed raw materials were protected from light and stored at room temperature until the day of experiments.

Supercritical carbon dioxide extraction with co-solvent. A factorial design of experiments and response surface methodology was performed to assess and optimize the impact of extraction operating conditions of astaxanthin from brown crab residues. The isolation of astaxanthin through supercritical fluid extraction was carried out following a full factorial design including 4 factors, 2 levels and 3 center points, which results in a total of 19 SFE experiments. From the literature [13–16] and some preliminary experimental results, process variables and their ranges equilibration time (0-30 min), extraction pressure (200-500 bar), extraction temperature (40-60 °C) and extraction flow rate (30–50 g/min) were chosen (Table 1). Other parameters have been fixed, such as the amount of sample (set at 25g), extraction time (set at 180 min) and ethanol content in the supercritical fluid mixture (set at 5 %, wt.). Three quantitative responses were investigated in this assay: total extraction yield (ηTotal), astaxanthin extraction yield (ηASX), and astaxanthin concentration in extracts (cASX). The extractions were carried out in a supercritical fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SFE-500F-2-C50). The extraction vessel was filled with 25g of dried crab residues and laboratory glass beads were placed on both endings of the cell, to achieve a uniform distribution of the solvent flow. Carbon dioxide and ethanol were delivered to the extraction vessel using two TharSFC P-50 high pressure pump (Thar Technology, Pittsburgh, PA, USA) until the desired pressure 200-500 bar. The mixture of solvents was preheated on a heat exchanger to a temperature of 40-60 °C. The pressure on the extraction vessel was maintained constant by an automated back pressure regulator (TharSFC ABPR, Thar Technology, Pittsburgh, PA, USA), which was located between the extraction vessel and the first fraction collector, with a total solvent flow rate of 30-50 g/min. After the extraction the solvent was evaporated off in a rotary evaporator at 40 ºC with a pressure of −0.58 bar, and the extraction yield determined. The resulting extract was kept at -20 ºC and dark environment until further analyses.

Astaxanthin content. The astaxanthin content of the extracts was determined through UV/Vis spectrophometry at a wavelength of 492 nm. The calibration curve for astaxanthin was linear within the range of 0 - 5 µg/mL using DMSO as solvent. All the extracts were previously dissolved in DMSO. The ASX extraction yield (ηASX) and ASX concentration in the extracts (cASX) were calculated and presented as µg of astaxanthin per gram of dried weight.

Experimental design analysis/statistical analysis. The results of the full factorial design, concerning the total extraction yield, astaxanthin extraction yield and astaxanthin concentration in extracts were analysed using the software MODDE, version 12, from Umetrics (Umeå, Sweden). Both linear and quadratic effects of each factor under study, as well as their interactions were calculated. Their significance was evaluated by analysis of variance. A surface, described by a second-order polynomial equation, was fitted to each set of experimental data points. First- and second-order coefficients of the polynomial equations were generated by regression analysis. The fit of the models was evaluated using the determination coefficients (R^2) and adjusted R^2 (R_{adj}^2).

Summary

Astaxanthin pigments derived from brown crab wastes were successfully extracted through the supercritical fluid technology. By using the statistical tool of the full factorial design and the response surface methodology, it was possible to evaluate the impact of process conditions on the recovery of astaxanthin. The extraction pressure, temperature and flow rate were the parameters that mostly affected the extraction of the natural pigment. The best operating conditions within the tested range to obtained astaxanthin-rich extract were achieved at 30 min of equilibration time, 500 bar, 40 ºC and 50 g/min of flow rate. Under these conditions, the astaxanthin content in the extract obtained was 1025 µg ASX/g which correspond to 5.18 µg ASX/g residue processed. The ASX extraction yield of SFE was 1.5-fold higher than traditional solid-liquid extraction. From the results obtained it can be concluded that supercritical fluid extraction technology can be considered as a good alternative to traditional solid-liquid extraction methods using organic solvents for the valorisation of marine crustacean waste streams, allowing their subsequent use in nutraceutical formulations and functional foods. Nevertheless, further studies should be addressed in order to evaluate the techo-economic feasibility of the all process as well as the life cycle of ASX.

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Antimicrobial properties of tannin extracts

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Introduction

Antibiotics are substances that kill bacteria or inhibit their growth and for this reason they were used as additives in the animal feed. After it was discovered that they also improve animal growth rates and consequently reduce production costs they were added to feed for non-therapeutic purposes as well. This practice was allowed for many years until a consequential increase in microbial resistance due to resistance genes transfer from animal to human strains of bacteria was discovered [1–3]. The use of antimicrobial compounds as growth-promoting factors after 2006 has been forbidden by the European commission with the Regulation of the European Parliament and of the Council (EC) No 1831/2003 [4]. Without the use of antibiotics, the incidence of infectious diseases is increasing, and alternative means to control bacteria growth are necessary [1]. Tannins are natural compounds with proven antimicrobial properties and as such represent suitable alternatives [5].

Experimental

The aim of our study was to determine the *in vitro* minimal inhibitory concentration (MIC) of tannins against *Escherichia coli* under different conditions. Various commercial plant extracts (quebracho, mimosa, colistizer and farmatan-chestnut extract), as well as two pure compounds (gallic acid and tannic acid) were studied. Minimal inhibitory concentrations of these compounds were determined using two complementary methods. Broth microdilution method was used, and MIC was determined by measuring optical density or by adding Iodonitrotetrazolium chloride (INT) dye irrespectively. The concentration of the media was also changed. Concentration of nutrients in the growth media was varied by changing the broth concentration, from half the concentration recommended by the producer, to double the concentration recommended by the producer.

Both methods used provided similar results. Initial concentration of bacterial culture on microtiter plates was 2×10^5 CFU/ml. Figure 1 shows MIC values obtained by measurement of optical density (OD) in media of different concentrations.

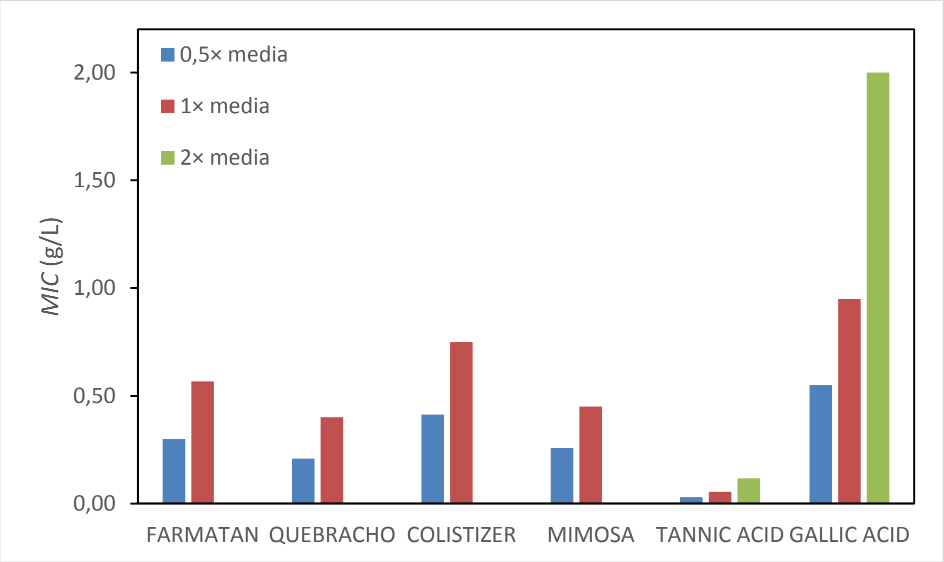

Figure 1: MIC values, obtained by optical density measurement, for different tannin containing extracts and broth concentrations. Where values are missing, the MIC determination was not feasible.

In Figure 2 MIC values of all the samples in broth of different concentrations obtained by INT dye addition are depicted. The results obtained by INT dye method are in good agreement with the results obtained by optical density measurement, while INT method also enables us to determine MIC in broth of double concentration recommended by the producer. Our results show that minimal inhibitory concentration (MIC) highly depends on both the selected compound and media concentration. Gallic acid has the highest MIC while tannic acid provides the best results (lowest MIC). MIC of all our samples is increasing roughly linearly with growth medium concentration, what may support the working hypothesis that tannins exert their antimicrobial effects by restricting the access of bacteria to their nutrients.

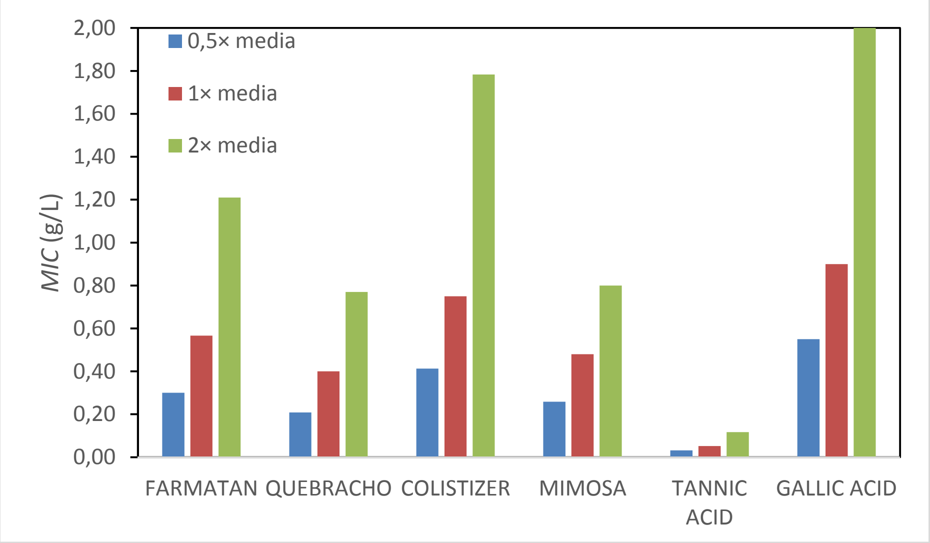

Figure 2: Comparison of MIC values (INT) among different media

Summary

MIC of all our samples is increasing roughly linearly with growth medium concentration. Tannic acid represents the best inhibitor of bacterial growth out of all tested compounds and extracts whereas gallic acid is the least effective.

Acknowledgement

This work is supported by the Ministry of Education, Science and Sport of the Republic of Slovenia through project grant AB FREE and by Slovenian Research Agency programme grant P2-0046.

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Development and economical analysis of aerogel production

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Abstract

The objective of this work is to develop a tool for the economical design of industrial aerogel production processes. Therefore, a physically sound model of the key process step, i.e. the supercritical drying, should be further developed and integrated as a MATLAB code into the widely used process simulation tool Aspen Custom Modeler. Besides the drying, the remaining parts of the process, namely gelation and solvent exchange, will be implemented. Production process for whey protein that is realized so far at lab scale only, is chosen as a model system and should be optimized with respect to its process and capital cost.

1 Introduction

Aerogels, which are a subject for research in the last years at the Institute of Thermal Separation Processes, can provide a tremendously large internal surface, low densities and excellent isolation properties. Therefore, they are suitable for numerous applications in pharmaceutics, material science and food industry. Because of their attractive characteristics, production of aerogels at an industrial scale is gaining more and more of importance. To evaluate the numerous research results of the last years from economical point of view, a tool to estimate the overall process- and capital cost is of high importance. The widely used program Aspen Custom Modeler was selected to build such a tool. The production of whey protein aerogels which is already realized at lab-scale in our Institute, was chosen as a model system. Using this model process (Fig. 1), the influence of the process parameters can be related with the structure of costs aiming at designing an effective and economic aerogel production process.

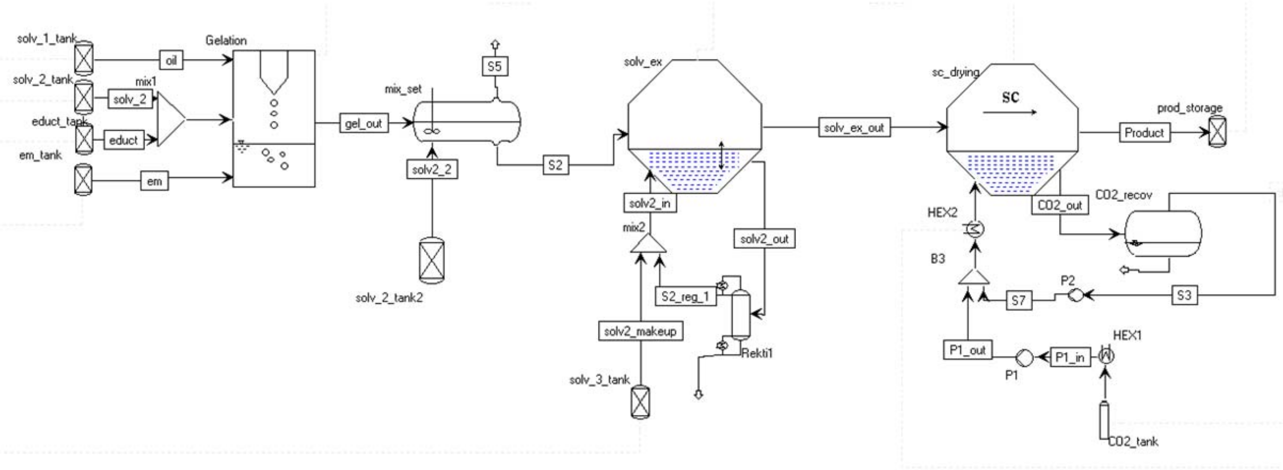

Fig. 1: Process flowsheet of the investigated whey protein production process

2 Results and Discussion

Currently, the energy and material balances of the whey protein production process are integrated into the tool. Sub-models for heat exchangers, pumps, tanks and flashes are implemented as well. To get a reliable tool, it is of great importance to accurately predict the physical properties of all components and mixtures involved. Therefore the Aspen internal databank is used in addition to our previous results (part of the PhD work of Ilka Selmer) [1]. As a starting point the supercritical drying is simulated to achieve the necessary energy and solvent consumption for this production step. Therefore a complex MATLAB model which predicts the drying time together with the concentration of the CO_2 outlet stream, was integrated in the ACM flowsheet simulation. Additionally thermodynamic equations for calculating Joule-Thomson-Effect, vapor-liquid equilibrium of CO_2/ethanol solutions (for CO_2 regeneration) and heat losses of the autoclave were derived and implemented. The first simulations of a standard drying ($p = 120\,bar, T = 40\,°C$, $\dot{m}_{CO_2} = 5\,g/min$) were done. The results are displayed in Fig. 2.

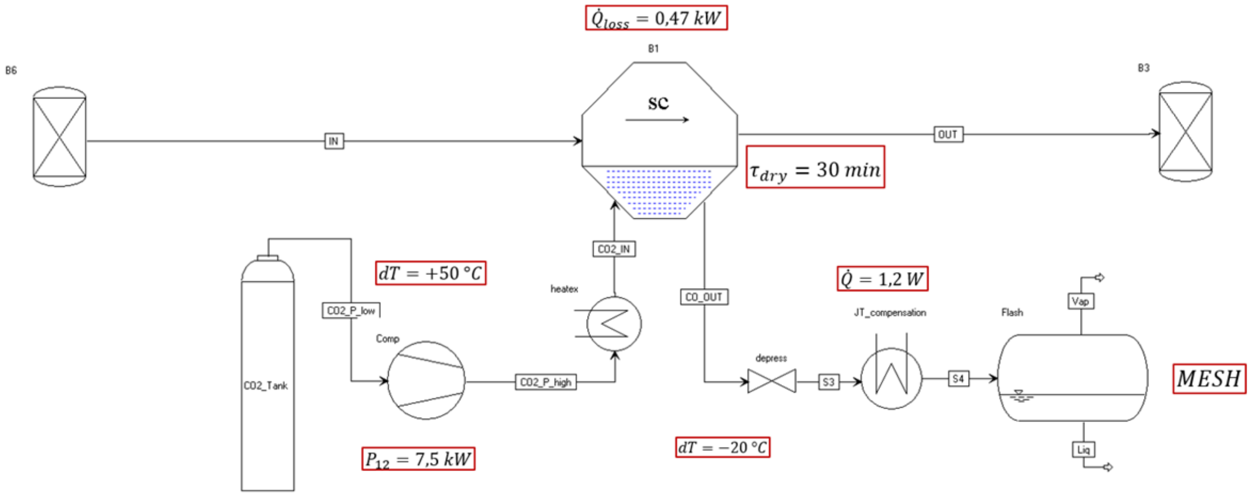

Fig. 2: Results of benchmark simulation

Using prices for solvents and auxiliaries available to us these calculations lead to an overall cost of 2.15 € per drying of 1 l of whey protein aerogels. This benchmark simulation will be used to show possible improvements such as recycling of used CO_2 after purification.

3 Summary

With the work performed a basis is laid for the primary goal of the project – to design an economically efficient aerogel production processes at various scales, from lab to industrial.

4 Acknowledgement

We are grateful to the Allianz Industrie Forschung (AiF) and the Forschungskreis der Ernährungsindustrie E.V. (FEI) for financial support (AiF 19712 N).

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Digitalisation and Remote Control of a Planetary Roller Extruder for use in the PGSS and Foaming Processes with compressed carbon dioxide

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Introduction

Against the backlight of digitalisation and energy revolution, the accurate acquisition and visualisation of all data generated by industrial equipment is becoming increasingly important in order to make continuous processes economically and ecologically more effective. Additionally, digitalisation enables the operator to access information about the plant at least in near real-time to determine whether steady operation conditions have been reached. Detailed measurement data also enable the development of predictive analytical models for the determination of optimal process parameters in regard to product quality and energy savings [1]. In addition, this data can be used to identify the most significant disturbance variables to ensure consistent product quality and develop advanced control mechanisms [2].

In this work, an existing plant consisting of a single screw extruder – called SSE – and a planetary roller extruder – called PRE – is to be modified in order to be used in the PGSS process (Particles from Gas Saturated Solution) for the preparation of fine-grained and spherical polymer particles for additive manufacturing.

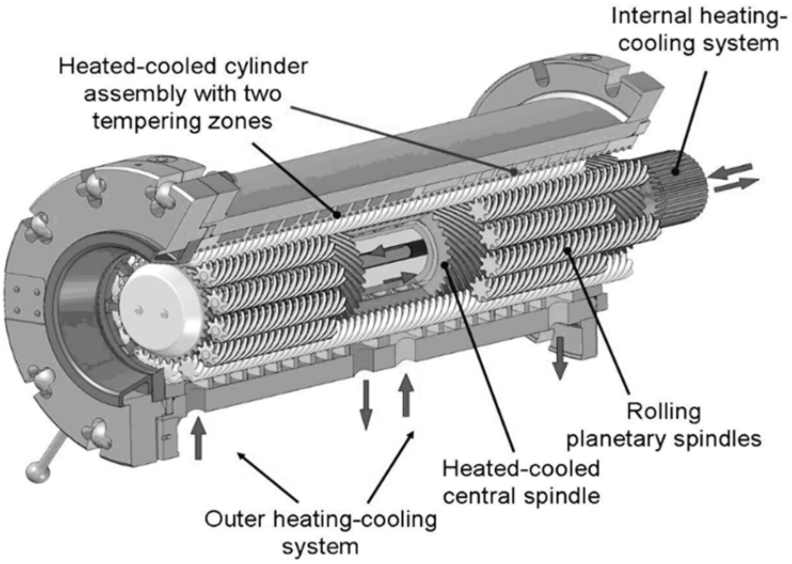

Fig. 1: Sectional drawing of a planetary roller extruder module [3]

A PRE module has several planetary spindles revolving around the main spindle, as shown in Fig. 1. Between the helically toothed flanks of the epicyclic gearings and the main spindle as well as those between planetary spindles and the cylindrical barrel the polymer melt is rolled out and transported along the axial direction, whereby intense mixing takes place. The temperatures of the PRE’s barrel cylinders as well as the temperature of the main spindle are controlled by separate heating circuits to enable precise temperature control during the whole process. [3] In comparison to SSEs or twin screw extruders and despite the PRE’s well known heating and mixing performance, little scientific work is published concerning the investigation, modelling or simulation of the process behaviour [4], especially in combination with the PGSS process.

In order to identify the most significant process variables and to correlate different process parameters with the resulting quality of the products, the chosen input values and resulting process variables have to be visualised and logged for evaluation, as small deviations can greatly affect product quality [5]. As the current design of the plant cannot do this, the current situation and the necessary retrofitting are described in the following paragraph.

Experimental

The present system, as shown in Fig. 2 and used for a poylmer foaming process, consists of the PRE, the SSE and the temperature control units – called TCUs – which are all controlled manually at different human-machine interfaces (HMIs). The SSE is used for melting the polymer granule and for the primary pressure build-up and receives the input values from the first HMI via the MODBUS RTU protocol. In the PRE the homogenisation of the mixture of polymer melt and compressed carbon dioxide is performed and the PROFINET technical standard is used for the communication with the second HMI, where the data is visualised. The TCUs control the temperatures of the PRE modules and main spindle, as shown in Fig. 1, and their connection to the third HMI is realised through the serial communication interface RS 485. The compressed CO_2 is taken as a liquid from the laboratory grid and pumped into the PRE at the end of the penultimate of the three modules. All these systems are unable to continuously log or provide the measured data, screw speed, temperatures and pressure, to an external system for later analysis of process parameters with regard to product quality.

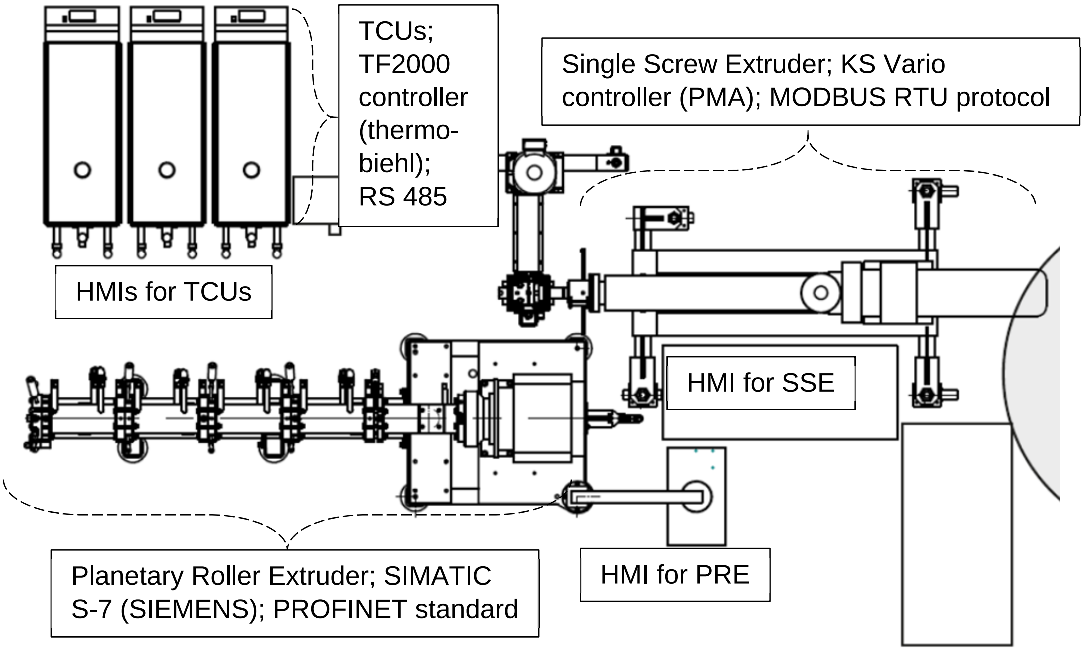

Fig. 2: Current setup of the extrusion plant; listing PLCs, HMIs and communication protocols

In order to overcome these limitations a higher-level PLC (Programmable Logic Controller) will be installed into which the existing PLCs will be integrated. This higher-level PLC uses the PROFINET infrastructure and is equipped with different communication modules to understand the different protocols used in the plant. The logging and visualisation on an external device will be realised over an OPC-UA server (Open Platform Communication – Unified Architecture) that is connected to a standard laboratory computer used for the visualisation and analysis of the data. The connection of the laboratory computer to the OPC-UA server and the visualisation of the data will be realised using the LabVIEW software and the Data Logging and Supervisory Control Module. Additionally, the sensors and actuators used in the control of the PGSS process will be integrated into the higher-level PLC and provided to the visualisation in the LabVIEW program via the same OPC-UA interface. This data will then be used to identify the most significant process variables, correlate process parameters with product quality and improve existing analytical models for the planetary roller extruder.

Summary

An existing extrusion plant, consisting of a traditional single screw extruder and a planetary roller extruder, both operated independent from each other, is retrofitted by integrating the existing PLCs into a higher-level PLC, which enables data logging and visualisation on an external laboratory computer. Additionally, by incorporating new

control loops based on the signals of the different parts of the plant the fail-safety of the system and reliability are enhanced and automated remote operation is achieved.

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Study of pressurized technologies to remove genetic material from porcine pulmonary arteries

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Introduction

The rate of organs annually transplanted increases by year. In 2016, 15.5 organs were globally transplanted per hour, a 7.25% of increase over 2015[1]. However, it still constitutes less than 10% of the transplant needs, which became the lack of compatible organs/ tissues in a social and health problem. Several strategies, e.g. regenerative medicine or synthetic scaffolds production has been deeply addressed with the aim to meet the organ/tissue demand. However, scientist community is specially interested in the bio-scaffold production alternative. This novel approach is based in the cleaning of a tissue/organ from cellular components (i.e. genetic material), which are the main cause of immune rejection, while keeping the extracellular matrix (ECM) functionality. Lately, the cleaned ECM is re-cellularized with healthy cells.

Nowadays, the most common methodology for ECM cleaning is based in a chemical-enzymatic treatment for long times (12-24 h approx.). The target is to force the cell lysis by disruption of the bilayer membranes to reach the genetic material (DNA), which would be respectively driven by detergents and DNases. Therefore, immune reactive material as well as lipids, which impair a correct re-cellularization of cleaned ECMs, would be removed. However, there is currently no efficient process to remove these compounds keeping intact the ECM and which prevents the toxic effect of the chemical's leftovers to the recipient organism.[2]

From last decade, the use of high-pressure carbon dioxide (as supercritical fluid or high-pressurized CO_2 mixtures) have been considered as a suitable alternative for detergents in the ECMs cleaning process[3]. CO_2 under high pressures are known to extract lipids from solid matrices and they are expected to be inert to the long proteins of the ECM. Moreover, its tuneable polarity, when mixing with cosolvents, could enhance the extractability of more polar components i.e. genetic material or immune reactive proteins. In addition, pressurized water extraction (PWE) results in a lower solvent and time consumption method to extract proteins and protein-like compounds from solid biological matrices.[4] High pressures and temperatures enhance the diffusivity and mass transfer of compounds while decreasing the viscosity and surface tension, which promotes water penetration into the solid matrix and allows less polar compounds solubilization.[4,5] The combination of both technologies could became a potential tool for 1) bilayer membrane disruption (because of detergent-like properties of pressurized CO_2) and 2) denaturalization and extraction of DNA (and other immune reactive protein-like compounds).

Here we present preliminary results of sole and combined pressurized technologies, i.e. high-pressurized CO_2 mixtures and PWE with/without enzymatic activity, as environmentally friendly, quicker and safer potential alternatives for the ECMs cleaning of porcine pulmonary arteries.

Experimental

Pulmonary arteries provided by the Thoracic Surgery research group (Lund University, Sweden), were cut into small pieces (5 mm^2 size) and kept at -80°C until use. Tissue pieces were individually or sequentially submitted to 1) high-pressurized CO_2-EtOH-H_2O, 2) pressurized water and 3) DNAse activity.

Disruption of cell membranes by pressurized CO_2 mixtures

A home-built equipment (Fig.1) was used for high-pressurized CO_2-mixture treatment. Tissues were carefully placed in a sample holder designed to treat biological samples.

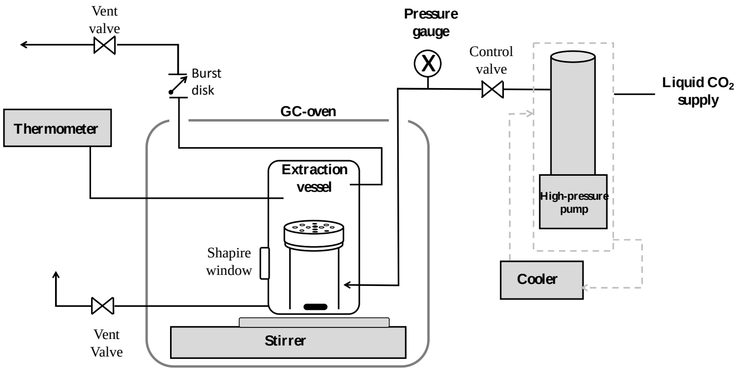

Fig. 1.: Scheme of the high-pressure system used for tissue delipidation. CO_2 in liquid state from a dip tube cylinder was pumped by a high-pressure pump.

Sample holder was loaded in a stainless-steel extraction vessel of 80 mL volume (Ångström Laboratory, Uppsala University, Uppsala, Sweden) connected to a thermocouple, two vent valves and the connection line to the liquid CO_2 high-pressure pump (Isco 260D, Teledyne Technologies Inc., NE, USA). Fluid stirring was achieved by placing the extraction vessel on top of a magnetic stirrer (VWR, Leuven, Belgium). A GC-oven (HP 5890 GC, Hewlett-Packard Co. CA, USA) was used to heat up the system at desired temperature.

Extraction vessel was pre-loaded with EtOH:H_2O (95:5, v/v) before loading the sample container. The treatment was carried out in static mode at 300 bar, 35°C for 30 min, after 1h of phase equilibrium.

Results showed that pressurized CO_2-EtOH-H_2O fluid (0.87 X_{CO2}) was able to disrupt cell membranes (lipid extraction, data not shown) but to remove genetic material (Fig. 2.). Moreover, the fiber linearity observed at both untreated and pressurized CO_2-EtOH-H_2O treated samples suggested that no drastic changes after treatment happened (Fig. 2.).

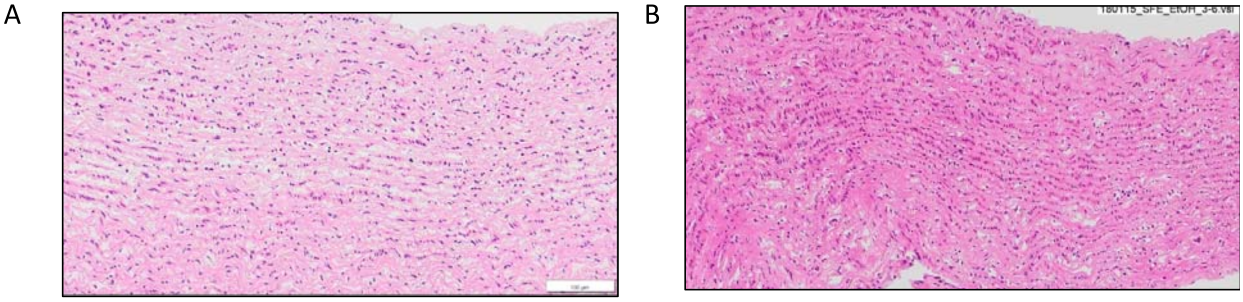

Fig. 2.: Eosin-hematoxylin staining results of A) untreated and B) pressurized CO_2-EtOH-H_2O treated pulmonary arteries. Purple areas correspond to DNA from nuclei.

Pressurized water extraction treatment

A screening of PWE conditions was performed by a one-factor-at-a-time methodology. Pulmonary arteries were submitted to the pressurize water treatment using an Accelerated Solvent Extraction (ASE 350) equipment. Two tissue pieces were loaded by treatment in a stainless-steel cell (10mL) filled with glass balls. Parameter such as temperature (RT, 40°C and 50°C), time per cycle (5 and 15 min) and number of cycles (1 and 2) (Tab. 1).

Tab. 1.: Details of the one-factor-at-a-time optimization for pressurized water extractions.

ID sample	Temperature (°C)	Time per cycle (min)	N° of cycles
1	40	5	1
2	40	15	1
3	40	15	2
4	RT	5	1
5	RT	15	1

6	RT	15	2
7	50	5	1

Obtained extracts (1 mg/mL) were analysed by UV-VIS absorption at 260 nm (characteristic for nucleic acids) and 280 nm (characteristic for proteins) using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Oslo, Norway). Ratios between both wavelengths were used for sample comparison. In addition, treated tissues were analysed by Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FT-IR) (Thermo Fisher Scientific Nicolete iS5, Oslo, Norway). The data were acquired in the spectral region 400-1800 cm^{-1} with a 4 cm^{-1} resolution and 16 measured scans per sample.

Results suggested that time and number of cycles exerted a moderate influence in the dsDNA removal. However, higher intensities for 260 and 280 nm were observed at increased temperatures. Although extracts obtained at 50°C showed higher absorbances compared to those at 40°C (Fig. 3A), a higher contribution of genetic material was found for the latest (as it is shown for 260/280 ratios, Fig. 3B). In addition, ATR-FT-IR profiles were similar for all extracts except for those obtained at 50°C which indicated qualitative changes (Fig. 3C).

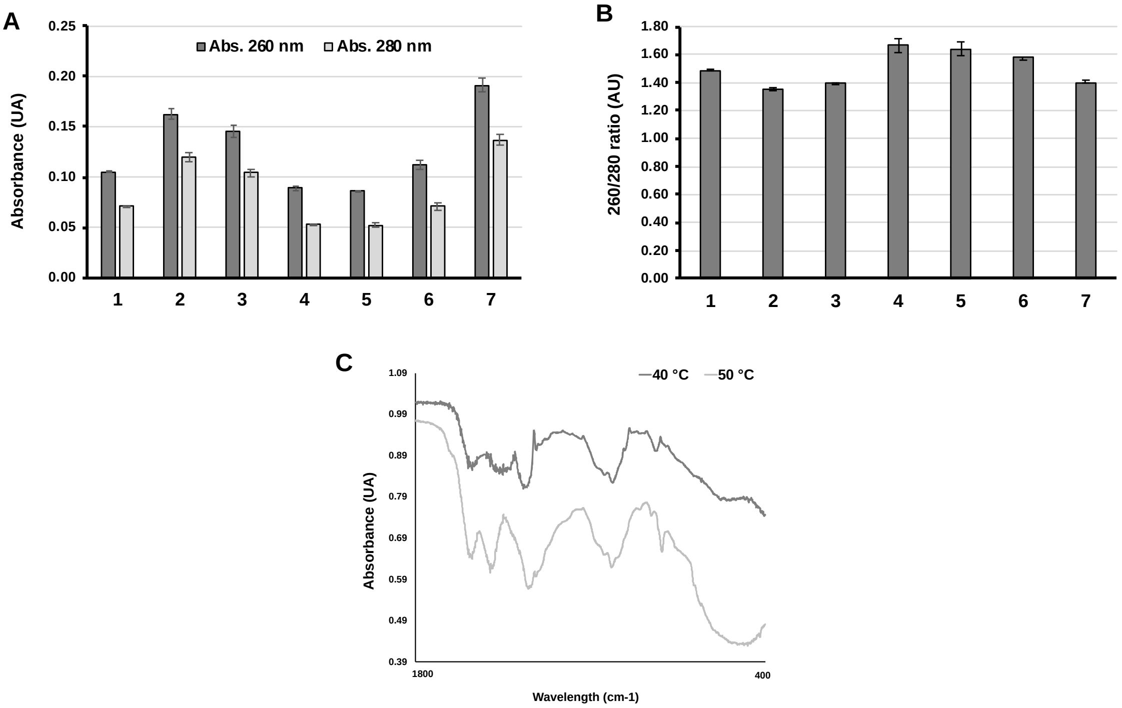

Fig. 3.: Extract composition of PWE extracts at different temperatures evaluated by A) absorbance at 260 and 280 nm, B) ratio 260/280 and C) ATR-FT-IR profiles. Numbers indicate the sample identification detailed in Tab. 1.

PWE technology resulted inefficient for a substantial removal of dsDNA, as suggested eosine-hematoxylin stained samples (data not shown).

Combination of pressurized technologies

Cell disruption achieved by pressurized CO_2-EtOH-H_2O treatment is expected to facilitate the subsequent enzymatic activity, because of the higher accessibility to the target. Therefore, the most suitable parameters were selected for the combined pressurized treatment, i.e. 300 bar, 35 °C and 30 min for pressurized CO_2-EtOH-H_2O followed by 2 cycles of 15 min at 40 °C for pressurized water. In addition, the effect of pressurize enzymatic-buffer (approx. 100 U/mL of benzonase at pH 8) in the dsDNA removal was evaluated at optimized conditions.

None of the combinations showed a successful dsDNA removal however, some colour changes in the stained tissue were observed for pressurized CO_2-EtOH-H_2O followed by enzymatic PWE.

Summary

PWE technique was slightly able to remove some genetic material depending on the temperature applied. A high dsDNA extraction was observed with the temperature however, over 50 °C changes in the tissue composition were observed. The combination of pressurized technologies such as pressurized CO_2-EtOH-H_2O and PWE resulted inefficient for a complete removal of genetic material from pulmonary arteries tissues. Similar results were obtained when tissue was submitted to pressurized CO_2-EtOH-H_2O followed by PWE-enzymatic treatment.

Acknowledgment

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Click synthesis and optimization of polyethylene glycol by click chemistry in supercritical carbon dioxide

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Introduction

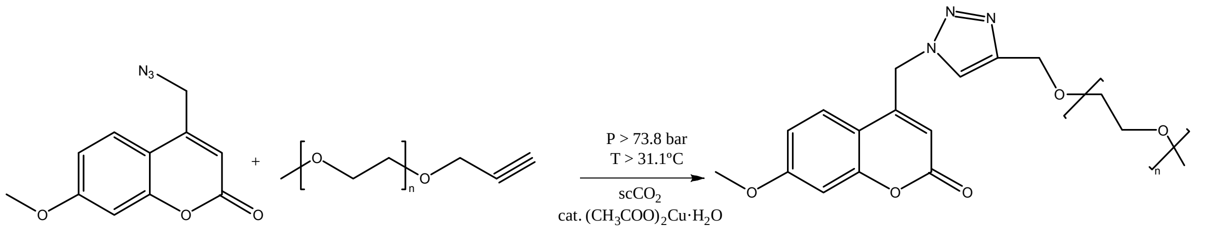
The growing demand for "intelligent" polymers has led to the continuous search for simple and efficient methods for their generation. Polymer-drug conjugates are finding increasing use as novel anticancer agents. Polymeric systems conjugated with a drug result in the controlled release of drugs as this occurs when a carrier material, specifically a polymer, is combined with an active principle so that it is released from the system in a pre-designed way. The release of drugs achieves more effective therapies as it eliminates the administration of both overdoses or doses below the minimum effective dose. In this context, click chemistry emerges as a simple and extremely powerful methodology due to its ability to easily and effectively interconnect different substructures. This has resulted in a wide range of applications in biomedical sciences, organic synthesis and materials science. Click chemistry has the advantage of being a highly realible methodology,clean, with excellent performance and compatible with a large number of functional groups. One of the most well-known reactions is copper(I)-catalyzed alkyne azide cycloaddition (CuAAC). Terminal alkyne group reacts with an azido group to form a thermally and hydrolytically stable triazole ring, where N, N-dimethylformamide (DMF) or tetrahydrofuran (THF) are the most common solvents used to achieve the conjugation of chemical product.This work aims to find an option that avoids the use of toxic solvents such as THF or DMF, and to use a solvent that can protect medicines from degradation and is additionally environmentally sustainable, using supercritical technology. In recently, considerable attentions have been focused on using supercritical carbon dioxide (scCO_2) as a reaction medium for organic reactions due to their attractive physical and toxicological properties.

This research focuses on the conjugation of a polymer, polyethylene glycol (PEG), with an active ingredient, coumarin, by means of click chemistry, carrying it out for the first time using supercritical technology without the use of a ligand being necessary for the reaction to be carried out satisfactorily. In order to carry out the reactions corresponding to click chemistry based on Cu catalysis, it is necessary that the polymers previously incorporate azide or alkyne groups on which to carry out the functionalization.

Results and discussion

The first objective was to obtain an active-polymer conjugate by means of a 1,3-dipolar cycloaddition reaction between azide and alkynes in $scCO_2$.

Previously the different reagents were synthesized to include the necessary groups, was begun by introducing the azide group to the active principle. There are several methods to introduce an azide group, but in general the aliphatic azides are synthesized through nucleophilic substitution of a halogen with an excess of sodium azide. The functionalization of the polymer by having the terminal group OH through the addition of Michael was introduced the propargyl group.



Scheme 1. Click reaction carried out in $scCO_2$.

The synthesis of conjugate coumarin with PEG was confirmed from ^{1}H NMR spectra as is shown in Figure 1. The protons of PEG chain were observed in the range of 3.5-3.8 and methoxy proton of PEG were

observed in 3.35. The triazole proton appeared as a singlet at 7.87. The aromatic protons of coumarin were observed with a doublet 7.45, 7.44 and 6.84, 6.86 and multiplet around 6.8. The presence of the chemical shifts of proton corresponding to PEG, triazole and coumarin confirmed that the conjugate has been successfully synthesized.

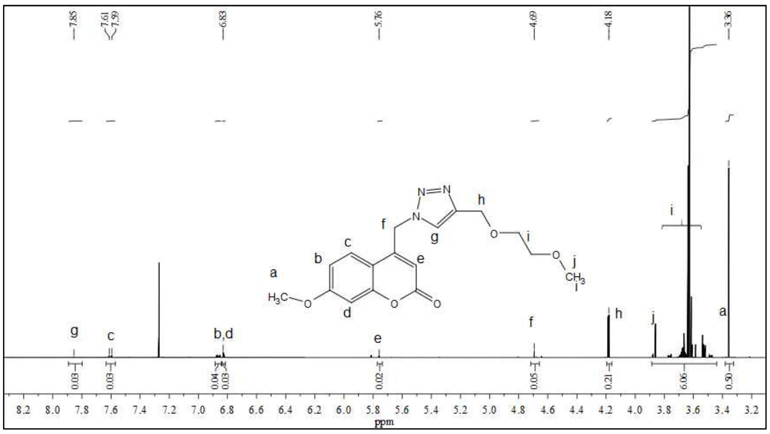


Figure1. ^1H NMR Spectra corresponding to a click product synthetized in $scCO_2$ for conditions of 110 bar, 37ºC, 24 h and catalyst load of 21%.

Once the feasibility of the click product formation process has been demonstrated using supercritical technology using CO_2 as the solvent, attention is paid to the different variables that influence the process such as the percentage of catalyst, pressure, temperature and reaction time. In order to optimize the click reaction for maximum yield.

Table 1. Evaluation of Pressure and load catalyst in CuAAC in $scCO_2$.

Sample	Pressure	Temperature	Load of catalyst	Density[1]	Yield
	bar	ºC	%	g/l	%
1	80	47	21	232.05	14.30
2	90	47	21	316.2	32.55
3	100	47	21	448	40.09
4	130	47	21	669.25	50.89
6	100	37	21	680.05	45.65
7	130	37	21	768.99	59.25
8	150	37	21	801.66	65.54
9	170	37	21	826.6	70.98
10	130	60	21	505.84	45.52
11	100	37	0	680.05	0
12	100	37	8,5		17.29
13	100	37	12		40.52
14	100	37	45		37.45

[1] Density of $scCO_2$ was determined from to Equazione of Bender.

In order to analyses the influence, the different variables on yield, the results obtain were compared. The result was shown in the Table 1. The PEG 2000 shows a low solubility in

$scCO_2$ at experimental conditions. The solubility increased with pressure, while it decreased with temperature likely due to the CO_2 density change which is related to the yield obtained.

Summary

The formation of the triazole ring is demonstrated by means of ^{1}H-NMR and MALDI-TOF mass spectroscopy. The functionality of the cycloaddition reaction of coumarin and poly-acid lactic acid in supercritical medium is therefore verified.

When the reaction was carried out in the absence of the cupric salt, as shown in Table 1, no products were observed which indicated that the presence of $(CH_3COO)_2Cu\cdot H_2O$ catalyst was very important.

Acknowledgment

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Inline Near-Infrared Spectroscopy for Continuous High-Pressure Processes

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Introduction

Diverse analytical methods are important and necessary to obtain information on a chemical substance, on a chemical reaction or on a system like a chemical plant in which several reactions can occur. With inline measurements, it is possible to analyze a system or a chemical reaction *in-situ*. With this technique, the time to get a result is usually short what makes it possible to react to a system's change. [1]

The free-radical polymerization of ethylene takes place at pressures between 1400 bar and 3500 bar and at temperatures between 130 °C and 330 °C. At those harsh conditions, it is challenging to apply inline measurements to get information on the reaction system *in-situ*. Hence measurements in those systems are often carried out offline, for example by taking and analyzing a sample. [2]

Spectroscopy with electromagnetic radiation is one possible way to analyze a high-pressure system inline by directing the optical path of radiation through a view cell filled with the high-pressure medium. The medium needs to be homogeneous for this method. In the past, inline analysis on high-pressure systems was already performed with the Fourier-transformation infrared (FTIR) spectroscopy with measuring times of seconds. [3-5]

IR spectroscopy belongs to vibrational spectroscopy. Any intramolecular vibration which changes the transition dipole moment is IR-active and can be determined by this technique. [1]

The raw data of an FTIR spectrometer are interferograms which can be Fourier-transformed to spectra. A background spectrum of an empty cell as well as a sample spectrum of the sample filled cell is necessary. Both spectra in relation to each other result in the sample's absorption spectrum. With the integrated Lambert-Beer law, it is possible to determine concentrations of substances with known integrated molar attenuation coefficient and known path length of the cell. For quantitative analysis, it is also necessary that a spectral line of interest does not overlap with another one. IR spectra are influenced by temperature and pressure. A higher temperature can result in shifted and lowered maxima and in broadened spectral lines for example. For quantitative analysis, this broadening effect can be considered by integrating spectral lines over a specific range. The independence of temperature and pressure has to be confirmed for each new measuring method. [1,6]

Experimental

The analysis system is constructed the following way: The radiation is routed out of the spectrometer with an optical fiber to a collimator. The collimator is used to spread out radiation parallel into space. A second collimator is placed opposite to the first one to collect the spread out radiation. It is then routed back through an optical fiber to the spectrometer where it reaches the detector. A view cell with NIR-radiation permeable sapphire windows is placed between the collimators. Thus the radiation can pass through the high-pressure medium. A exploded assembly drawing of the cell is shown in figure 1.

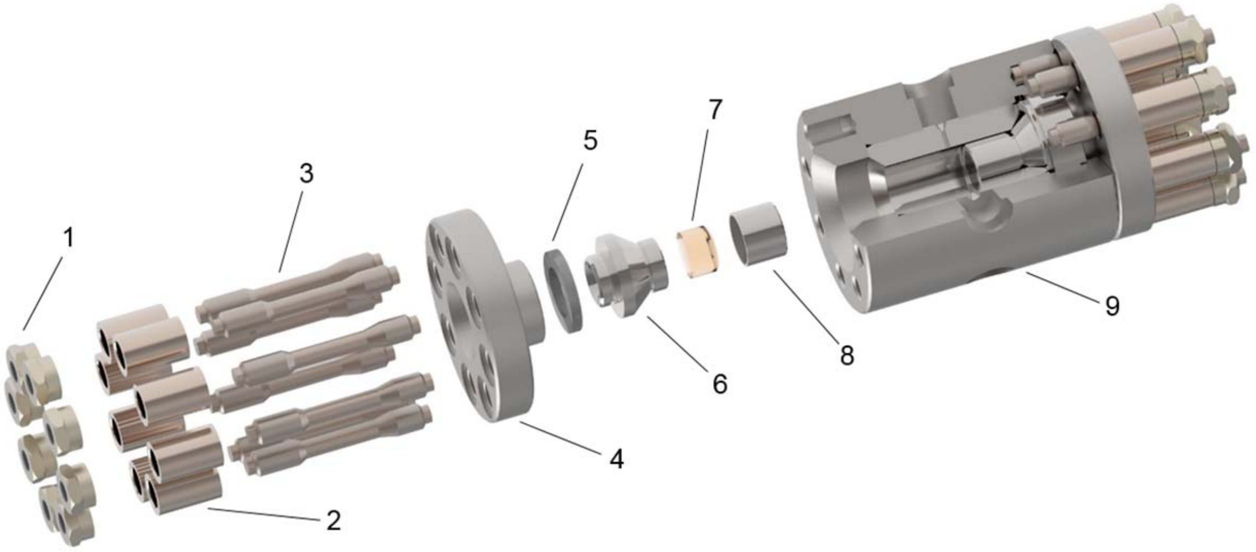

Figure 1: Exploded assembly drawing of the view cell with nuts (1), casings (2), tension screws (3), flange (4), spacer (5), sealing cone (6), NIR-radiation permeable sapphire window (7), sapphire cap (8) and body (9).

The cell can withstand pressures up to 3000 bar and temperature up to 300 °C. The path length is adjusted by lengthening the sealing cones of the cell. To prevent an additional residence time due to the view cell, a modified cap is designed. This cap should conduct the high-pressure medium through the cell like a tube. It is possible to perform inline measurements in the near-infrared range between 10000 cm^{-1} and 4000 cm^{-1} and in continuous operation mode with this setup. [7]

In batch experiments, absorption spectra of pure ethylene are obtained at different temperatures and pressures. The spectral line of the first overtone of ethylene is integrated from 6500 cm^{-1} to the maximum at around 6140 cm^{-1}. The half-band integrated intensity *B* can then be determined by the integrated Lambert-Beer law. The half-band integrated intensity is independent of temperature and pressure over a certain temperature and pressure range. The values are compared to those in literature. [3,4]

During a batch polymerization of pure ethylene at 200 °C and 2000 bar, the progress of the spectral lines in figure 2 is investigated by inline-FTIR analysis. The spectral lines of polyethylene are increasing because of its formation and those of ethylene are decreasing because of its conversion. The isosbestic point at which the absorbance does not change during the reaction could be observed at 5865 cm^{-1}. [5]

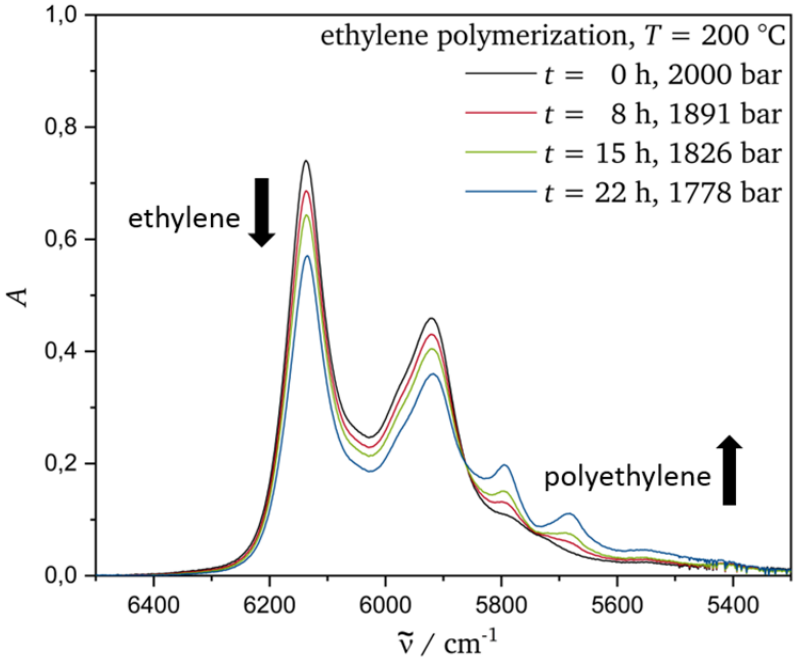

Figure 2: Absorbance *A* of the first overtone of ethylene and polyethylene during a batch polymerization. The isosbestic point is obtained at 5865 cm^{-1}.

Spectra of the reaction system were taken at steady state conditions with different amount of oxygen during continuous polymerization. Oxygen acts as an initiator. Hence, higher amounts of oxygen result in higher conversions of ethylene. Thus the spectral lines of ethylene are decreasing and those of polyethylene are increasing with higher oxygen content. The conversion of ethylene is determined by three different methods using IR spectra. These results are compared to the conversion determined by taking a polymer sample.

Summary

An FTIR spectrometer was extended to an analysis system that makes it possible to perform inline measurements of a high-pressure medium in continuous operation mode. The half-band integrated intensity of the first overtone of ethylene was determined at different temperatures and pressures to confirm the method’s independence of temperature and pressure. The isosbestic point could be verified for a batch polymerization of ethylene. During continuous polymerization, the conversion of ethylene was determined by three different methods using FTIR spectra and then compared to the gravimetric conversion.

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Graphene nanoribbon formation inside carbon nanotubes

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Introduction

Coronenes are planar π-conjugated molecules, which can form coaxially stacked coronene columns inside carbon nanotubes held together by π-π interactions and nanotube-coronene interactions without forming covalent bonds [1] [2].

Low-temperature filling is ideal for encapsulating coronene in carbon nanotubes because at high temperature (above 400°C) coronene derivatives, not soluble in common solvents, are formed on the outer surface of the carbon nanotubes (CNTs) [3] [4].

With the use of supercritical CO_2 ($scCO_2$), showing low viscosity, absence of surface tension, and low solvation effect, high diffusive approach can be reached at 50ºC and 150 psi. Low-temperature filling with common solvents used for the encapsulation of apolar molecules does not result in filling ratios as high as with the use of sCO_2, besides, common solvents can get trapped in between the filler molecules [5].

Coronene molecules have been used as precursors for graphene nanoribbon (GNR) formation in the nanotube inner cavity. By increasing the temperature coronene can be turned into dimers and other linearly condensed coronene oligomers regarded as short GNRs (s-GNRs); further polymerisation/conversion into long GNR (L-GNR) can be induced by heating the samples at 700ºC [4]. It is possible to follow these reactions by Raman and photoluminescence spectroscopy to study the formed nanoribbons.

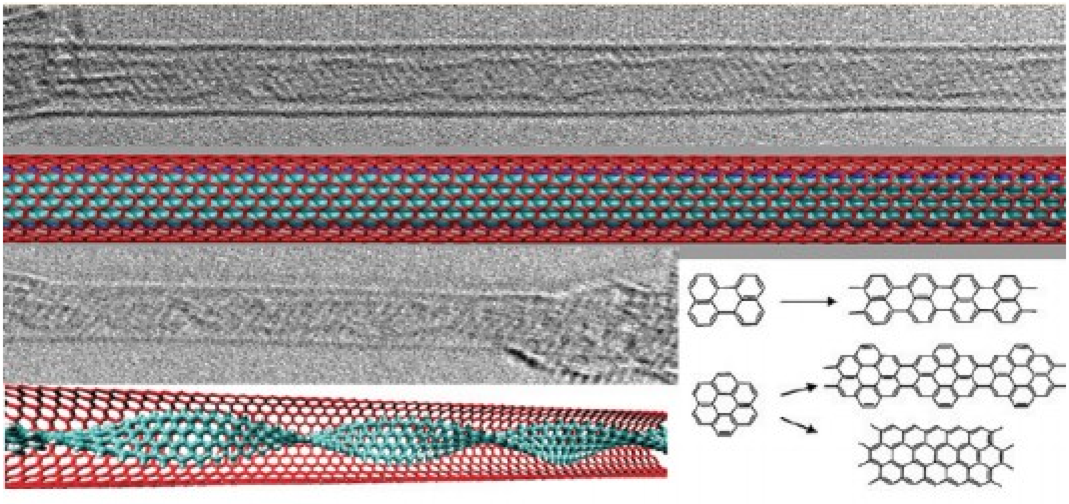

Figure 1 Graphene nanoribbons encapsulated in single walled-carbon nanotubes [6].

Experimental

Opening tube caps.

Single-walled carbon nanotubes (P_2 Carbon Solutions, Inc., average diameter 1.4 nm) were heated at 570°C for 20 min.

$ScCO_2$ filling

Immediately after opening their caps CNTs were mixed with coronene and introduced in an $scCO_2$ reactor at about 150 bar and 50°C for 96 h.

Cleaning the products

Coronene@CNTs were stirred in toluene for half an hour and filtered using PTF membrane. The washing liquid was analysed by UV spectroscopy to detect coronene. The procedure was repeated until the coronene content in the washing liquid was less than 1 mg/L.

Fabrication of Graphene Nanoribbons

The nanoribbons were prepared by heating Coronene@CNT in vacuum at temperatures starting at 450°C for 12 hours, then increasing in 50-100°C steps. A sample was taken and characterized at each temperature.

Raman characterisations

The annealed samples were analysed with the lasers 785, 633, 532 and 325 nm to observe the nanoribbons and other coronene derivatives. Multiple lasers were needed because of different resonance conditions of different nanoribbons.

Photoluminescence

PL spectra of the graphene nanoribbons were measured in D_2O solution, using deoxycholate (DOC) as dispersant.

Summary

Coronene molecules can be encapsulated in the interior of carbon nanotubes; when increasing the temperature, the enveloped coronene molecules are converted into graphene nanoribbons with increasing length which can be detected by Raman spectroscopy and can be further studied by photoluminescence spectroscopy.

Acknowledgments

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Supercritical CO_2 extraction of antiproliferative ingredients from natural sources

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Introduction

The increasing evidence establishing a positive correlation between diet and a reduced risk of chronic illnesses such as diabetes, cardiovascular diseases, cancer, among others, has driven the growth of nutraceutical and functional food sectors[1]. The market for these natural and naturally derived products is expected to continue growing in the years to come, which will certainly generate profit potential for the industries involved[2,3].

Extraction is the first step for separation of the natural ingredients from the original raw materials. In this field, supercritical fluid extraction has revealed to be a particularly interesting technology for the isolation of bioactive products. In fact, this technology offers many advantages over conventional extraction methods, thus allowing to overcome some limitations of the latter[4].

Within this context, the main goal of this work was to develop adequate methodologies by using supercritical fluid technology, for the isolation of different bioactive molecules with promising anticancer effect, namely terpenes, isothiocyanates and ω-3 fatty acids. The studied natural matrices included aromatic plants (*Lavandula* and *Mentha* genus), vegetables (watercress) and fish-processing residues (sardines). All natural extracts were characterized in terms of chemical content and their bioactive effect, namely antiproliferative activity, was evaluated using a human colorectal adenocarcinoma cell line.

Experimental

Raw material. *Lavandula angustifolia*, *Lavandula stoechas*, *Mentha spicata*, *Mentha piperita* var. *citrata* and *Mentha pulegium* were collected in Mafra, Portugal, and dried at room temperature, in the absence of light. Watercress was kindly provided by Vitacress Portugal and dehydrated using a freeze dryer at -40°C. Canned sardine (*Sardina pilchardus*) heads and offal were kindly provided by Fábrica de Conservas A Poveira and dehydrated using a freeze dryer at -55°C. All raw materials were milled using a chopper and the particle size of the ground material determined (ranging from 250μm to 1000μm).

Supercritical CO_2 (SC-CO_2) extractions. Extractions were carried out in a supercritical fluid extraction system (SFE-500F-2-C50, Thar Technology) comprising a 500mL cylinder extraction cell and two different separators, each of them with 500mL of capacity, with independent control of temperature and pressure. The extraction and pre-treatment conditions applied to each raw material are summarized in Table 1.

Table 1. Extraction and pre-treatment conditions applied to each raw material.

Raw material	Pressure (bar)	Temperature (°C)	Solvent	Flow rate (g/min)	Time (min)
Aromatic plants	250	50	CO_2	20	15 + 60
Watercress	250	35		10	120
Pretreatment with humidified raw material	Patm to 250	25 to 35		-	0 to 120
Canned sardine residues	300 to 550	35 to 75		25	135

Extracts characterization. Aromatic plants' extracts were characterized by GC-MS. Mass spectrometry detection was performed in SCAN mode and samples were examined from m/z 29 to 299. Watercress' extracts were analysed by HPLC-DAD after applying the derivatization protocol described by Budnowski et al. (2013) and adapted by Rodrigues et al. (2016)[5,6]. PEITC peak was detected at 284 nm. Glycerides contained in sardines' extracts were converted in methyl esters following the transesterification method described in the NP EN ISO 5509 2003 and analysed by GC-FID[7]. Fatty acid methyl esters were identified by comparison of relative retention times in the samples with those obtained for a standard mixture.

Cell based assays. Cell culture of human colorectal adenocarcinoma cell lines (HT-29 and Caco-2) and cytotoxicity and antiproliferative experiments were performed as previously described by Rodrigues et al. (2016)[6]. All cell-based experiments were performed in triplicate using at least three independent assays. Cell viability was determined using PrestoBlue® viability reagent, Cell Titter® aqueous one solution cell proliferation assay or colorimetric 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay, and expressed as percentage of viable cells relative to the positive control. Effective concentration values (EC50) were obtained from the dose–response curves.

Results

SC-CO$_2$ extractions from aromatic plants. Five supercritical CO$_2$ extracts were obtained from plants from the genus *Lavandula* and *Mentha*, which consisted mainly of terpenes. Their major components, which can be typically found in essential oils, are shown in Table 2.

Table 2. Major compounds found in each aromatic plants' extract.

	L. angustifolia	***L. stoechas***	***M. spicata***	***M. piperita* var. *citrata***	***M. pulegium***
Major compounds	Linalool Camphor Borneol Linalyl acetate	Camphor Fenchone Eucalyptol	Carvone	Linalool Linalyl acetate Eucalyptol	Pulegone Menthone Menthyl acetate

Figure 1 shows the EC50 values (concentrations required to decrease cell viability in 50%) obtained for each fraction. Overall, *L. angustifolia* exhibited the highest antiproliferative effect followed by *M. spicata* and *L. stoechas* extracts. The antiproliferative activity displayed by extracts is probably related with their phytochemical composition, since linalool, camphor, fenchone, carvone and linoleic acid, among others, have demonstrated cytotoxic activity against several tumor cell lines[8-10]. Nevertheless, it is also possible that the exhibited activity was due to a synergistic or additive effect between the different components of extracts.

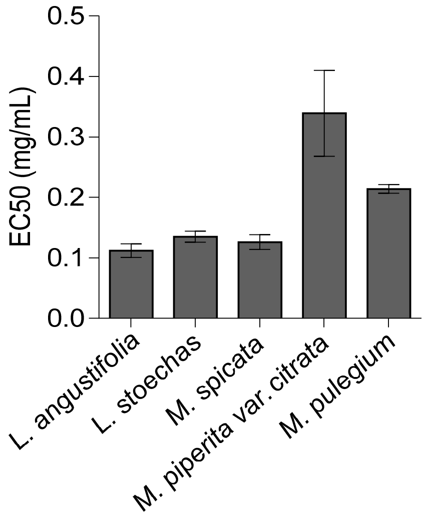

Figure 1. EC50 of *Lavandula* and *Mentha* extracts on HT-29 cells after a 24h treatment with non-cytotoxic concentrations.

SC-CO$_2$ extractions from watercress. Since isothiocyanates (ITCs) are obtained through enzymatic hydrolysis from glucosinolates (GLs), preliminary pre-treatment experiments were performed on watercress, in an attempt to maximize the conversion of GLs in ITCs by action of endogenous myrosinase. The impact of pressure, temperature, incubation time and moisture content (125, 250, 900% of water dry basis) in GLs' hydrolysis was studied. Yields of phenethyl ITC (PEITC), the main ITC contained in watercress, ranged from 1.0±0.2mmol/g$_{\text{dry watercress}}$ to 29.3±2.6mmol/g$_{\text{dry watercress}}$. Among all conditions tested, an incubation period of watercress humidified with 125% of water (dry basis), during 60 minutes at 35°C and atmospheric pressure, showed to be the best pre-treatment condition to improve the extraction yield of PEITC. Additionally, considering this same pre-treatment condition, a significantly higher concentration in PEITC was achieved when supercritical CO$_2$ was used as solvent, when compared with the results obtained after a conventional solid-liquid extraction with hexane.

In order to evaluate the antiproliferative effect of watercress' extracts, a human colorectal adenocarcinoma cell line (HT-29) was subjected to treatment with non-cytotoxic concentrations of extracts for 24h. Results showed that all extracts were able to inhibit HT-29 cell proliferation, with EC50 values similar to PEITC standard (EC50=27.8±1.9mM). This indicates that their bioactivity is mainly related with the presence of PEITC, which has already been reported to inhibit HT-29 cells growth[11].

SC-CO_2 extractions from canned sardine residues. SC-CO_2 extraction was explored to extract lipids, with special focus on ω-3 fatty acids, from sardine residues. The impact of pressure and temperature on the global yield of each extract was studied and compared with the results obtained after Bligh and Dyer (B&D) extraction[12]. Table 3 shows the experimental conditions at which SC-CO_2 experiments were carried out, as well as the global extraction yield obtained for each experiment. Extraction yields ranged from 19.95g/100$g_{dry\ residue}$ (at 55ºC and 300bar) to 27.4g/100$g_{dry\ residue}$ (at 75ºC and 550bar). Considering the yield obtained by B&D extraction as reference, at higher pressures (425 and 550 bar) SC-CO_2 was able to produce extracts with similar or even higher yields than those obtained by a conventional B&D extraction. In particular, the yield obtained at 300bar and 75ºC was 52.48% higher than the one achieved by other authors for freeze-dried sardine heads, at optimal conditions of 300bar and 75ºC[13].

Table 3. Extraction conditions applied to sardine residues and respective global extraction yields.

Sample	Temperature (°C)	Pressure (bar)	CO_2 density (g/mL)	Global extraction yield (g/100$g_{dry\ residue}$)
SC-CO_2 1	35	300	0.93	20.10
SC-CO_2 2		425	0.98	25.81
SC-CO_2 3		550	1.02	26.98
SC-CO_2 4	55	300	0.85	19.95
SC-CO_2 5		425	0.92	24.63
SC-CO_2 6		550	0.97	26.84
SC-CO_2 7	75	300	0.77	21.80
SC-CO_2 8		425	0.85	23.97
SC-CO_2 9		550	0.91	27.41
B&D extraction	Room T	Atmospheric P	-	24.99

The major components of sardine extracts were palmitic acid (C16:0), DHA (C22:6 (n-3)), oleic acid (C18:1 (n-9)), EPA (C20:5 (n-3)), myristic acid (C14:0), cetoleic acid (C22:1 (n-11)), stearic acid (C18:0), palmitoleic acid (C16:1 (n-7)), gadoleic acid (C20:1 (n-11)) and cis-vaccenic acid (C18:1 (n-7)). These results are in accordance with those reported in the literature for sardine extracts[13,14].

In order to evaluate the antiproliferative effect of sardine extracts, tests were conducted in HT-29 cells (Figure 2). Extract *SC-CO_2 4* (55°C, 300bar) highlighted for its capacity of highly inhibiting HT-29 cell proliferation (EC50=0.45±0.06mg/mL), while the B&D extract showed the lowest antiproliferative activity (EC50=2.87±0.53mg/mL) on the same cancer cell line.

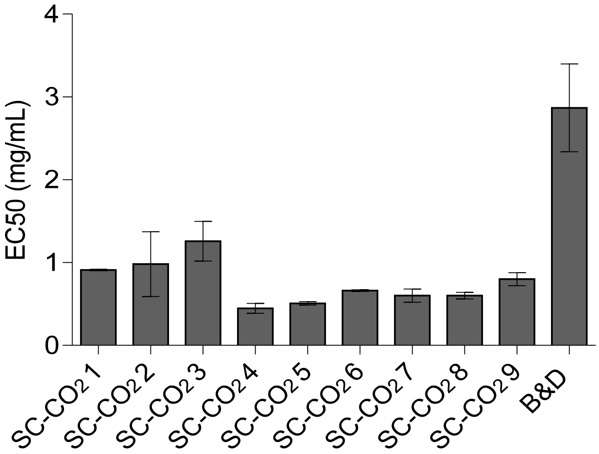

Figure 2. EC50 of sardine extracts on HT-29 cells after a 24h treatment with non-cytotoxic concentrations.

Summary

Supercritical CO_2 extraction proved to be an effective technology for the isolation of bioactive-rich fractions, from different plant and animal raw materials, with promising antiproliferative effects on colorectal adenocarcinoma cells.

Acknowledgment

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Characterization and Modelling of JetCutting Process for the Continuous Production of Spherical, Biopolymer based Aerogels

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Introduction

Aerogels being known as the lightest solid materials have a tremendous potential in a wide range of applications, where high pore volume and high surface area play major roles. The low densities (< 0.1 g/cm^3), high porosities (up to 99%), high specific internal surfaces (up to 1200 m^2/g) and the open-pore network structure are extraordinary properties of aerogels, which make them usable materials in many different areas. Because of their excellent insulating properties, they are used as thermal insulation materials (Beatens et al., 2011) and silencers (Forest et al., 2001). Aerogels can also be used as chemical sensors (Plata et al.,2004), filter systems (Amonette et al., 2017) and catalysts (Wang et al., 2017) as well as in environmental remediation as adsorbers for heavy metals, oil and organic compounds for water treatment and in food or pharmaceutical products as functional foods or active substance carriers with targeted drug release (Betz et al., 2012, Selmer et al., 2015,Tkalec et al., 2015, Maleki et al., 2016, Ahmadi et al., 2016, Goimil et al., 2017).[1-11] Aerogels are a unique class of materials since their preparation heavily relies on supercritical fluid technology and thus cannot be thought as purely chemical synthesis: it necessarily requires the technological knowledge. The overall scheme involves three steps: (I.) formation of a gel through different mechanisms; (II.) solvent exchange; (III.) supercritical drying with carbon dioxide at high pressure (~ 120 bar). A high porosity and specific surface area as well as a defined internal pore size distribution are desired product properties.

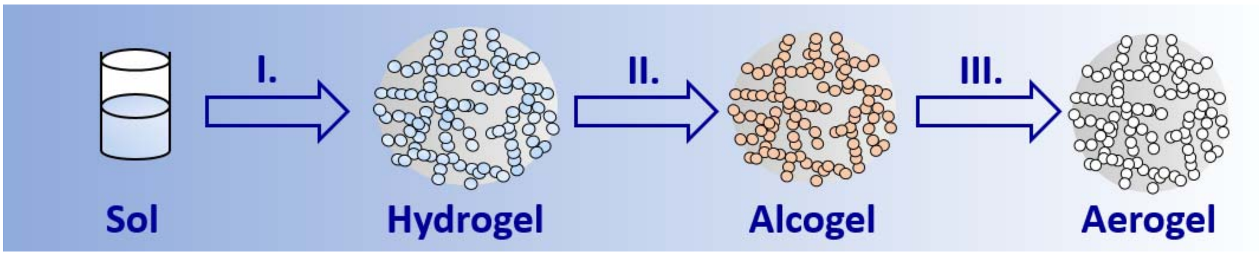

Fig.1: Basic steps of aerogel synthesis.

In order to reduce the process time and to decrease the overall process costs, the fast manufacturing and drying of gels in form of spherical particles is in the focus.

In principle, two methods are used for the production of aerogel particles in spherical form, whereby the applied method is chosen in dependence of the desired particle size. The production of particles in the size range from 500 µm to 2 mm is commonly carried out by gelation during spraying or dripping processes (Deze et al. 2012, García-Torres et al. 2016, Yu et al. 2017, Prüße et al. 2000, Sescousse et al. 2011).[12-16] Although these methods are established for laboratory-scale production, they are not suitable for industrial-scale production due to the low production rate.

In first publications it was shown, that spherical alginate gel particles can be continuously produced with high throughput (approx. 10 kg/h) and narrow particle size distribution in the size range from 500 µm to 2 mm (Prüße et al. 2000) via jet cutting method.[15] Here, a liquid jet of the solution to be gelled is generated and - with the aid of a fast-rotating knife - cutted into drops of the according size. These drops fall into a liquid gelation bath, so that spherical gel particles are formed. The size of the droplets depends on the frequency of the rotating knife and the nozzle diameter and thus the diameter of the liquid jet. The shape of the droplets is determined among other things by the drop height (Prüße et al. 2000), the surface tension of the gelling solutions and by the jet velocity.

Within the scope of this work, the knowledge about the production of different aerogel material systems (alginate, cellulose, silica) via jet cutting process shall be put on a broader base. Namely the range of accessible particle diameters should be extended and the physical as well as chemical influences on particle size and shape should be quantified. Narrow particle sizes and high sphericity are desired attributes, which are influenced by jet cutting parameters as well as the properties of the gelation bath (temperature/solvent/pH value). Based on the results, predictive models for the production of spherical beads and for different materials systems will be implemented.

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The influence of extracts from natural materials on the metabolic activity of human melanoma cells (WM-266-4)

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Introduction

Induction, growth and progression of cancer are multi-stage events and numerous studies have shown that various nutritional factors affect the cancer rate and thus prevent malignancy. Fruits, vegetables and herbs contain many different nutrient and non-nutrient phytochemicals. Groups of secondary plant metabolites; antioxidants, phenols and flavonoids are often found in various fruits, vegetables and herbs. It has been proven to provide protection against oxidative stress due to oxidizing agents and free radicals. Most herbal infusions, which are often used as domestic medicines, have antioxidant and pharmacological properties associated with the presence of phenolic compounds, especially derivatives of phenolic acids and flavonoids. Studies show that a change in nutritional behavior, such as increased consumption of fruits, vegetables, herbs, spices, pellets and whole grains, is a practical strategy for significantly reducing the incidence of chronic diseases. A key factor is maintaining the balance between oxidizing agents and antioxidants in order to maintain optimum physiological conditions in the body. Excessive production of oxidants can cause an imbalance leading to oxidative stress. Oxidative stress can cause oxidative damage to large biomolecules such as proteins, DNA and lipids, which increases the risk of cancer and cardiovascular disease. People and all animals have complex antioxidant defence systems, but they are not perfect, and therefore oxidative damage occurs. In order to prevent or slow down the oxidative stress caused by free radicals, sufficient amounts of antioxidants should be used. Since prevention is a more effective strategy than the treatment of chronic diseases, constant supply of phytochemical ingredients is essential to provide a defence mechanism to reduce the risk of chronic diseases. For this reason, in our work, we wanted to determine preliminary the best conditions for extracting the material (horsetail and rosemary) in order to achieve high levels of antioxidant content. The main goal of our work has been to check the correlation between the content of antioxidants, proanthocyanidins and total phenolics in the selected natural materials (horsetail and rosemary) and the reduction of the metabolic activity of skin cancer cells. [1–8]

Experimental

Influence of temperature and extraction time on the antioxidant activity (AA) of solutions of extracts has been evaluated by the radical DPPH method.[9–11] Design-Expert 7.0 software has been applied to determine the experimental plan and to process the data. The WM-266-4 cells have been grown according to the procedure and the prepared extract solutions were added to the cells in 8 different concentrations; c =100 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 20 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 10 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 5 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 1 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 0,1 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 0,01 $\frac{\mathrm{mg}}{\mathrm{mL}}$, 0,001 $\frac{\mathrm{mg}}{\mathrm{mL}}$. *In vitro* tests of metabolic activity (MA) have been done by WST-8 viability method. [12]

Summary

The DPPH analysis showed high AA of extracts obtained from horsetail and rosemary. Rosemary water extract showed higher AA, up to 91 % (the extract was prepared at an extraction temperature of 96 ° C). The influence of the extraction time was almost negligible, while the temperature was considered an important parameter.

- <u>Antioxidative activity of rosemary – preliminary measurements:</u> Design Expert proposed a square transformation: $AA = -79{,}79 + 3{,}69717 \cdot T - 0{,}020033 \cdot T2$ Based on the response equation it has been figured out that the time in the case of rosemary extraction does not represent an important factor, therefore the program removed it. The variance analysis (ANOVA) of our experimental results showed the significant fitting of our model (F = 637.90 and p <0.0001), which means that the model's adapting mistake is negligible. External influences have been marked as insignificant, therefore they do not significantly affect the change in the model and the results. The assumed quadrangular Pearson coefficient (before R2) has a value of 0.9872, which is at acceptable limits compared to the recommended R2, which is 0.9907. For the coefficient of precision we obtained a ratio of 52.109, which is more than 4, which is the minimum required value.

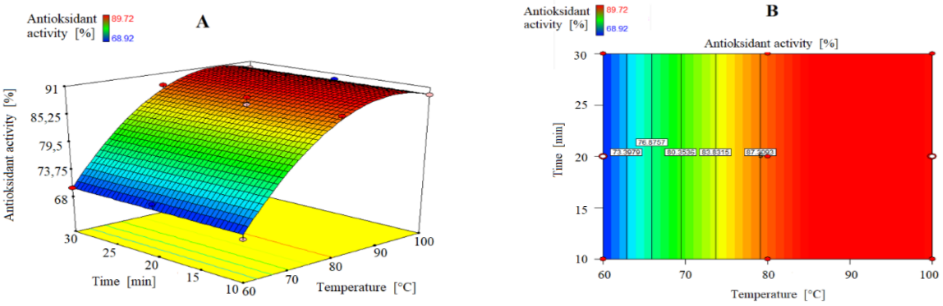
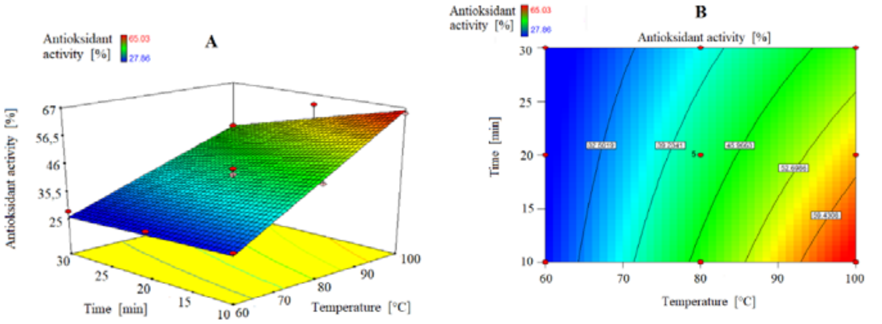


Fig. 1: Three-dimensional response surface (A) AA of rosemary extract as a function of temperature and time (B) two-dimensional contour diagram.

Fig. 1 demonstrates that the optimal extraction temperature was 96 ° C (90.79% AA was achieved).

- Antioxidant activity of the horsetail – preliminary measurements: Design Expert proposed a linear transformation: $AA = -37{,}14282 + 1{,}11775 \cdot T + 0{,}92433 \cdot t - 0.017712 \cdot T \cdot t$ The response equation shows that time and temperature have interactions. The variance analysis (ANOVA) showed the significant fitting of our model (F = 68.92 and p <0.0001). The inadequacy of the model mismatch is also irrelevant (p = 0.1532). The grounded quadrangular Pearson coefficient (before R2) has a value of 0.9057, which is within acceptable limits compared to the recommended R2, which is 0.9444. For the measurement coefficient, we obtained a ratio of 26.220, which is higher than allowed. As can be seen from Fig. 2, we did not achieve optimal points because of too large temperature range, so we did another experiment. We measured the AA after three, five, seven and nine minutes, as shown in Fig. 3.



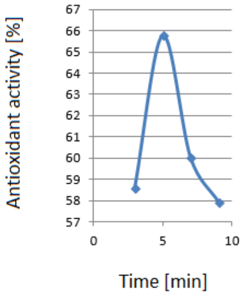


Fig. 2: Three-dimensional response surface (A) AA of horsetail extract with respect to temperature and time and two-dimensional contour diagram (B)

Fig. 3: Chart of antioxidative activity of the extract of the horsetail in relation to time.

Fig. 3 shows that the highest AA (65.79%) is achieved after five minutes.

- Antioxidants, total phenols and proanthocyanidins: The total phenolic content is calculated as the equivalent of gallic acid in mg per gram of the selected material (Fig. 4A). [10] The contents of the proanthocyanidins in mg per gram of material selected (mPAC / g material) are shown in Fig. 4B and the extract solution concentrations required for 50% inhibitory efficacy are shown in Fig. 4C.

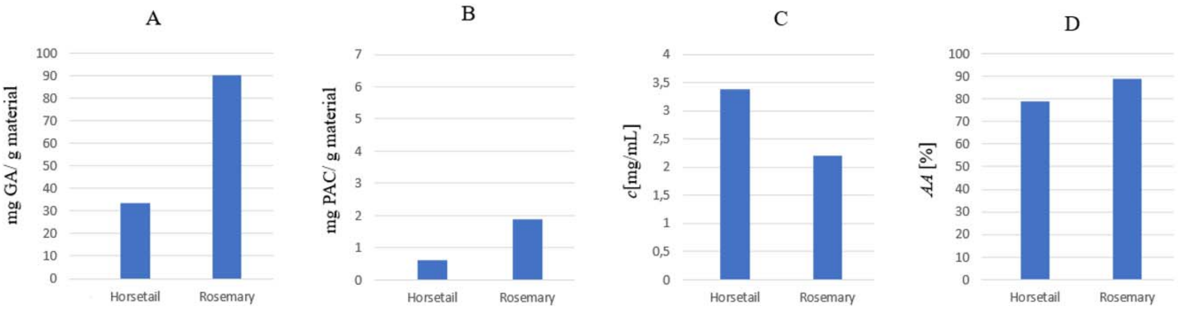


Fig. 4: A: Total phenolic content in selected extracts. B: Proanthocyanidin content in selected extracts. C: Concentrations of extracts required for 50% inhibitory efficacy. D: AA in selected extracts under optimal conditions.

Morphological changes of WM-266-4 cells, exposed to different concentrations of tested extracts and the control are shown in Fig. 5. Horsetail extracts inhibited metabolic activity (MA) of WM-266-4 cells; at a concentration of 100 mg/mL, MA dropped below 20% (Fig. 6A). For all lower concentrations, the values of MA were higher, but still below 50%. Rosemary extracts at concentrations of 100 mg/mL, 20 mg/mL, 10 mg/mL and 5 mg/mL significantly inhibited MA of WM-266-4 cells compared to control (Fig. 6B).

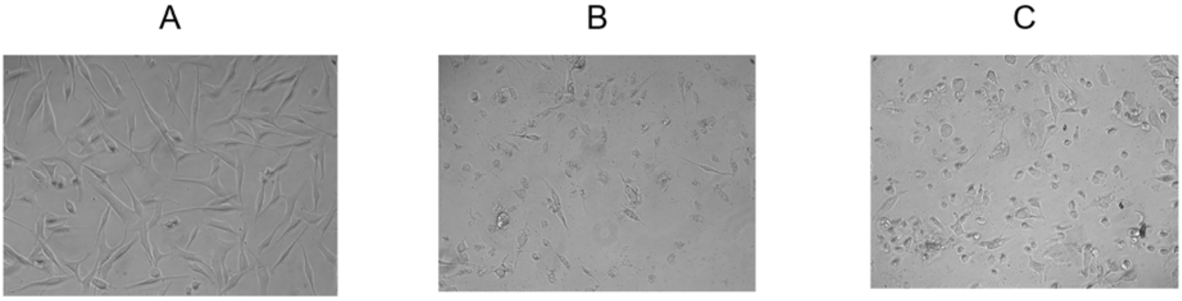


Fig. 5: Morphology of WM-266-4 cells after exposure to; A: the medium (control) B: the extract of horsetail (100 mg/mL) C: the extract of rosemary (5 mg/mL). Magnification 200x.

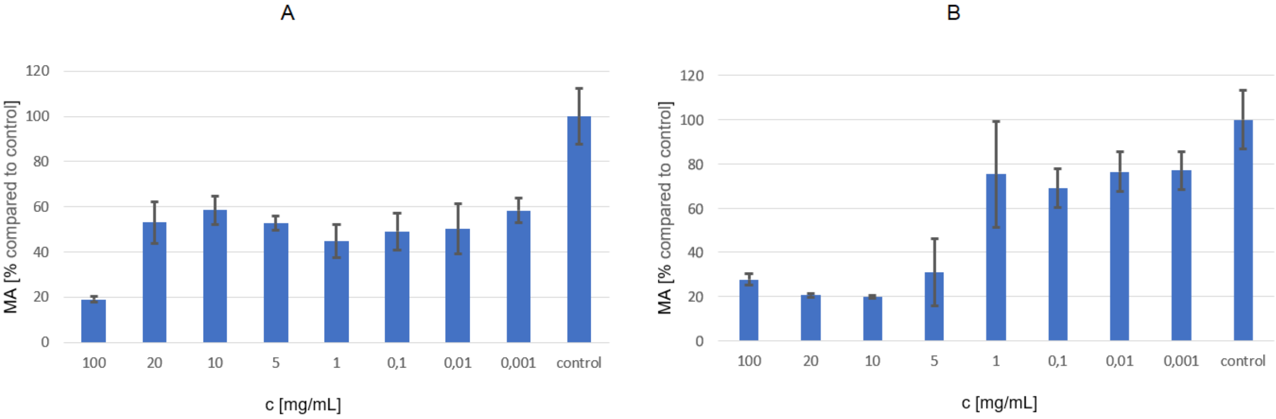


Fig. 6: The percentages of the metabolic activity of cancer cells versus control at different concentrations. A: horsetail extract, B: rosemary extract.

There is also a slight correlation between AA, proanthocyanidins and total phenolics. Higher proportion of proanthocyanidins and phenolics ((mg) / (g material)) contributed to a higher AA. Rosemary extract was efficient in inhibition of the metabolic activity of WM-266-4 cells at concentrations ranging from 100 mg/mL to 5 mg/mL. The metabolic activity of the cells decreased to 20 %. We anticipate the high value of antioxidants (89%) and total phenolics (90.10%). It has been determined that an extract solution with concentration 2.2 mg/mL is needed for a 50 % AA. Horsetail extract with a concentration of 5 mg/mL exhibited 79% AA. An extract solution with concentration 3.68 mg/mL is needed for a 50 % AA, whereas the content of PAC is 0.60 %.

Acknowledgment

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High-Pressure Ethene Copolymerizations for Functionalized LDPE Copolymers

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Introduction

The properties of polyethylene can be adapted by adding comonomers to the polymerization. While some comonomers are able to improve the properties by being incorporated into the copolymer, bifunctional comonomers can be used to adapt the properties in a second reaction. This two-step process allows a wide variety of properties to be amended.[1] Before new comonomers can be implemented into the process, they are tested on miniplant scale to determine different kinetic parameters. The reactivity ratios of the copolymerization of ethene and propargyl methacrylate, an example of such a bifunctional comonomer, are determined in a high-pressure batch setup. Small samples of polymer (up to 3 g) for analysis can be generated using a microinjection batch system. These can be used to gain first estimates of the reactivity ratios.

Experimental

The batch experiments are carried out at a temperature of 190 °C and a pressure of 2000 bar. The reactor is heated to injection temperature and pressurized with ethene. The comonomer and initiator are injected via the microinjection system. Once the solution is prepared and filled into the injection loop, it is pressurized with ethene to a higher pressure than the reactor. Using this overpressure, the solution is injected into the reactor and increases the pressure to the target pressure in the reaction. A simplified flowsheet of the setup is shown in figure 1. The reactor is designed for 3000 bar and 300 °C.

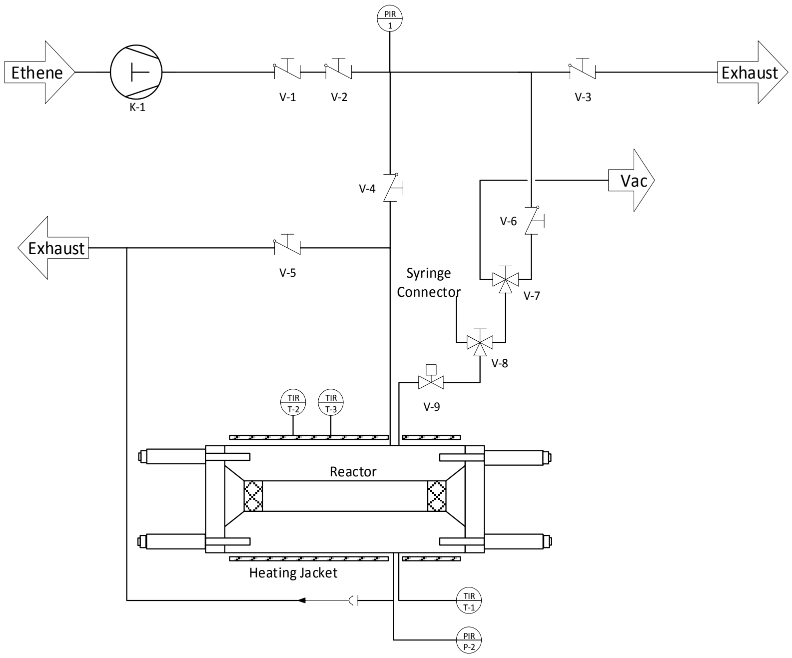

Figure 1: Flowsheet of the high-pressure microinjection batch setup.

Furthermore, the copolymerization is conducted in continuous miniplant experiments to compare the results of the batch and continuous setup. These experiments are carried out in a reactor with a volume of 15 mL, which is designed for 2500 bar and 300 °C.

The generated samples are analyzed by infrared and nuclear magnetic resonance spectroscopy to determine the polymer composition.

Summary

First approximations show that the reactivity ratio of the comonomer is larger than the reactivity ratio of ethene. The linear methods by *Mayo* and *Lewis*[2], *Fineman* and *Ross*[3] and *Kelen* and *Tüdös*[4] are used to determine the reactivity ratios. Furthermore, a non-linear fit is used to approximate the parameters. All determined values are used to generate the corresponding *Meyer-Lowry*[5] plot. These are used to compare the experimental data points to the theoretical progression of the reaction mixture composition with increasing conversion. It is observable, that the reactivity ratios determined by non-linear fit and *Mayo-Lewis* method describe the experimental data to a high degree.

Acknowledgment: SABIC

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Development of a new method for the separation and determination of vitamin D and its metabolites using ultra-high performance supercritical fluid chromatography-tandem mass spectrometry

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Introduction

Cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2) and their hydroxylated and ester metabolites constitute an important group of fat-soluble vitamins involved in calcium homeostasis, bone metabolism and other important pleiotropic functions in different tissues and organs of human organism [1]. Many studies have pointed out the relationship between the deficiency of these compounds and the development of metabolic bone diseases as, for example, rickets or osteomalacia. Recently, it has also increased the interest in the possible association of this fact with other kind of problems such as cardiovascular disorders, autoimmune and infectious diseases, diabetes and other chronic diseases related to liver and pancreas or even the development of several types of cancer [1, 2].

The metabolism of vitamin D in humans to their hydroxylated derivatives has been widely studied. Nowadays it is perfectly known that this vitamin can be synthesized from its precursor (7-dehydrocholesterol) in the skin by the effect of ultraviolet B radiation and also can be obtained by dietary intake and absorbed in the intestine. Then this inactive form is transported through blood to the liver and kidney where it is metabolized to the active 25-hydroxyvitamin D (25-OHD) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), respectively. However, other alternative routes are also developed in different parts of the organism to obtain those or other hydroxylated forms such as 24,25-dihydroxyvitamin D (24,25-$(OH)_2$D), 23,25-dihydroxyvitamin D (23,25-$(OH)_2$D) or their epimeric forms that present less activity than 25-OHD or 1,25-$(OH)_2$D [1, 2].

Despite all these studies there is still a lack of knowledge to other alternative routes, as occur with esters forms whose metabolism pathway has been little studied, or other metabolites taken by oral treatment in order to prevent or cure important chronical diseases.

For this reason, new studies that allow understanding more precisely the functions of the different metabolites of this kind of fat-soluble vitamins is of great interest for the scientific community. In this sense the simultaneous evaluation of the whole range of metabolites as well as the possibility of determining them even at very low levels at which some of these compounds are present in different organism tissues would provide a more consistent knowledge of this issue [1, 2].

Different kinds of immunoassays have been applied for the evaluation of Vitamin D in clinical samples, however these techniques are not able to distinguish between cholecalciferol and ergocalciferol forms and they suffer from cross-reactions due to the similar structures. That is why chromatographic techniques including high performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC) or supercritical fluids chromatography (SFC), specially coupled to mass spectrometry have been proposed as an excellent alternative for the individual determination of this group of compounds [3]. Among them, SFC has been proposed as an interesting green alternative due to its particular features.

This technique is based on the use of a highly compressible fluid near or above its critical temperature and pressure as mobile phase. This fluid is usually carbon dioxide (CO_2), which is an inert, readily available and cheap solvent with low toxicity and with an accessible critical point [4]. In addition, the combination of CO_2 with more polar co-solvents such as alcohols, allows increasing the range of the analytes with different polarities that can be evaluated simultaneously and the thorough control of the main variables that affect the separation (i.e. modifier nature and concentration, temperature, pressure and flow) becomes SFC into an excellent strategic for the separation of enantiomers forms. These two aspects are of great interest in the case of the evaluation of Vitamin D derivatives. Apart from that, the combination of this versatile technique with mass spectrometry (MS) provides the possibility of developing very sensitive methods with an unequivocal identification of the target analytes.

Based on above-mentioned, we propose the development and optimization of a new method based on the application of SFC coupled to tandem MS using a triple quadrupole as analyzer for the separation and determination of vitamin D and its metabolites.

Experimental

With the aim of developing a new methodology to the analysis of the most relevant vitamin D compounds, vitamin D_3, vitamin D_2 and nine different metabolites have been selected for this study due to their role in important pleiotropic actions carried out in different tissues and organs of human organism.

SFC analyses are performed in a 1260 Infinity II SFC System coupled to a 6495 triple quadrupole mass spectrometer both from Agilent Techonologies. Separation is carried out using a Torus 1-aminoantracene column (100 mm x 3 mm, 1.7 µm) from Waters Chromatography and different mixtures of CO_2 and methanol as mobile phase.

Initially, an exhaustive study of the type of ion source as well as the rest of MS parameters has to be carried out in order to achieve an adequate ionization and detection of all selected analytes.

After the optimization of the chromatography separation, SFC conditions (i.e. back pressure, column temperature, injection volume and modifier addition) will be optimized using an experimental design. The wide range of polarities of the evaluated analytes as well as the presence of epimers make necessary the exhaustive study of the different conditions applied in order to obtain de correct separation of the analytes. Then, the ion source parameters will be also optimized as well as the make up solvent used, in order to improve the sensitivity of the methodology.

Finally, the whole method will be validated in terms of sensitivity, linearity as well as repeatability in order to guarantee the reliability of the obtained data. We plan the application of the proposed methodology for the evaluation of vitamin D metabolites in biological samples in the next step of the project.

Summary

In this work, it is proposed the development of a new methodology based on the application of SFC as separation method followed by identification and quantification of a group of eleven fat-soluble vitamins using tandem mass spectrometry. The method is optimized including SFC separation as well as detection conditions, using an experimental design, and subsequently validated.

After analytical method development we plan to carry out its application to the evaluation of the content of the selected compounds in biological samples of different nature including plasma as well as liver and kidney tissues.

Acknowledgment

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Processing of Polymers for Composites and Advanced Materials

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Abstract

New techniques, capable to improve the properties of polymeric materials and the environmental compatibility of their manufacturing processes, are receiving a large attention by researchers in the field of material science and chemical engineering. The object of this part of the study was to examine the influence of the surface pre-treatment on shear strength of adhesive joint samples of the AlMg3 alloy for which the epoxy resin was used. The surface of the samples was activated mechanically, as well as chemically. The adhesion strength of the bonded samples was determined by measuring the shear force. On the basis of the obtained results, the best pre-treatment of the surface of the AlMg3 alloy was selected, and then it was examined how the type of crosslinker and the filler addition have the influence on the strength of adhesion. Glass and cotton fibers were used as fillers.

Mechanical pre-treatment in combination with chemical affects the change in the morphology of the surface of the sheet and contributes to the enhancement of the strength of adhesion. The type of crosslinker has an effect on the strength of adhesion. Adding filler adversely affects the properties of the adhesive joint due to the increased viscosity of the adhesive and it is heavy application on the surface of the sheet.

Polymer adhesives for vehicle’s parts: Introduction

Generally, the automotive industry is addressing the problem of mass reduction in many ways, among other things using thin sheets of high strength material. [1] Aluminium and its alloys have an advantage over other materials due to favorable physical and mechanical properties. Perhaps the most familiar feature of aluminium is small specific density, as well as corrosion resistance, high design ability, non-toxicity, quick and easy processing, attractive appearance, recyclability.

Theoretically, gluing is bringing two surfaces to a sufficiently close distance. However, this is impossible in practice, therefore an adhesive agent is the formation of links between the two surfaces. Before applying the adhesive to the surface, surface must be prepared or activated. [2,3] In doing so, the film oxide of aluminium is removed from the sheet metal, which contributes to the increase in the adhesion strength, while at the same time the smooth surface is porous, after which the adhesive enters the pores and connects the adherents better. [4] During adhesion, the adhesive is required to be fluent for the purpose of uniform covering and surface milling. It is also necessary to fill the uneven surfaces and cavities of the connecting surfaces. [5,6]

Modern adhesives are often based on epoxy resins, which are one of the most important types of thermosetting polymers. For them, the presence of two or more final epoxy groups per molecule is characteristic of the uncrosslinked state, while within the molecule there are hydroxyl groups. Some of the most important properties of the epoxy resins are: high chemical resistance; good adhesiveness to a large number of different materials; small volume changes during the hardening process; good physical and mechanical properties are sustainable and at elevated temperatures; possibility to cure in a large time and temperature range. [7] Epoxy resins are liquid, and their useful properties and characteristics of polymeric materials take only after crosslinking.

Experimental

The surface of the samples was activated mechanically, by grinding using SiC abrasive grinding paper (P240, P400 and P1000), as well as chemically, by base and acid (5 wt.% NaOH and 30 wt.% HNO_3). A reference sample was tested without pre-treatment, three samples were treated with various abrasive papers, and three samples were treated with various abrasive papers in combination with chemical pre-treatment.

Adhesive based on epoxy resin was prepared. Commercial epoxy resin was used, and crosslinkers and fillers were varied. As crosslinkers were used commercial crosslinkers based on amines (crosslinker GL2 based on polyfunctional diamine and crosslinker L based on cycloaliphatic amine). According to the manufacturer's recommendation, the ratio of epoxy resin and crosslinker was 60:40. Fillers were added in some formulations, where were used glass or cotton fibers. After adding components were homogenized. Mixing time for all formulations was five minutes according to manufacturer's recommendation. After mixing adhesive was applied in a thin layer on prepared surface. Surface of adhesive joint was constant in all samples. After application of adhesive and joining surfaces force by flat plate was applied.

Results and discussion

First, surfaces were treated by three different abrasive papers, which are with unbrushed surface shown on Figure 1. After brushing on sheet's surface were made drawings, which intensity depends on abrasive paper (smaller granulation of abrasive paper gives more intensity and roughness). It was noticed that after the chemical treatment in the case of all three granulations, the drawings from the grinding were lost, so that the surface morphology had changed. The results showed that the chemical pre-treatment contributed to the increase shear strength in all samples compared to mechanical preparation only, what is explained by changes in morphology. Mechanical pre-treatment by abrasive paper P1000 in combination with chemical pre-treatment were chosen for further researching.

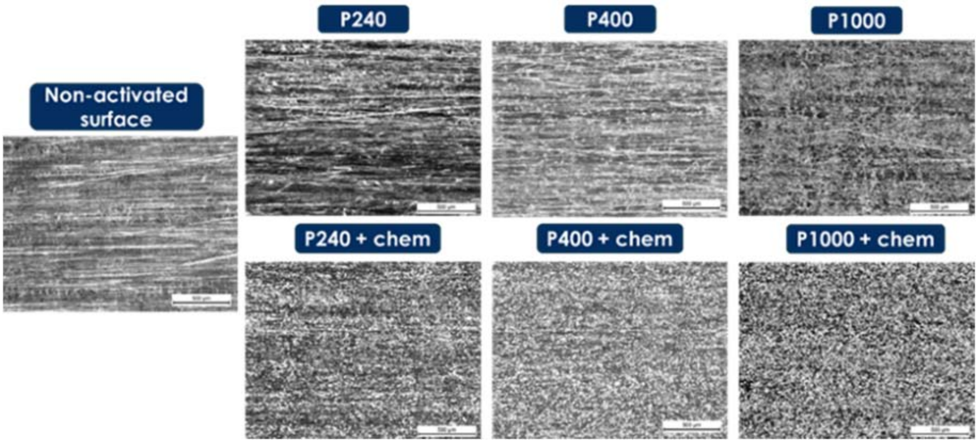
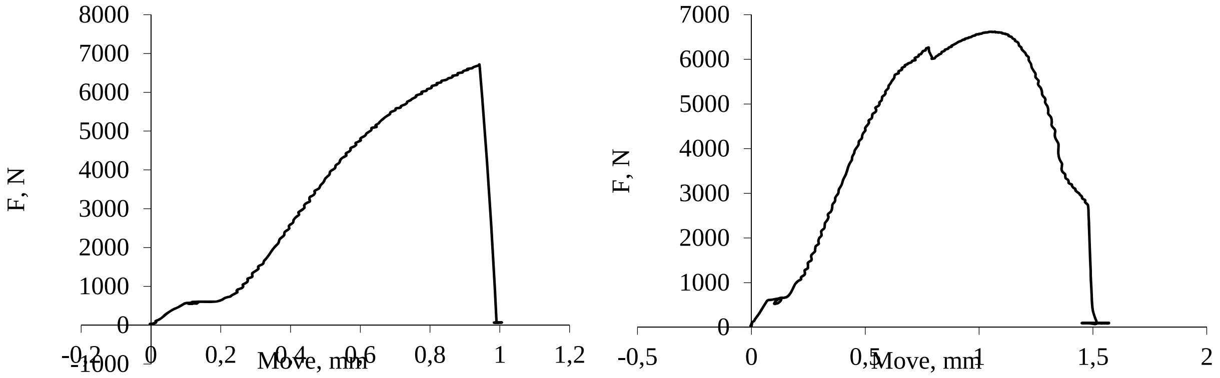


Fig. 1. Activated surfaces. Stereo microscope Leica 205A. Increase 40x.

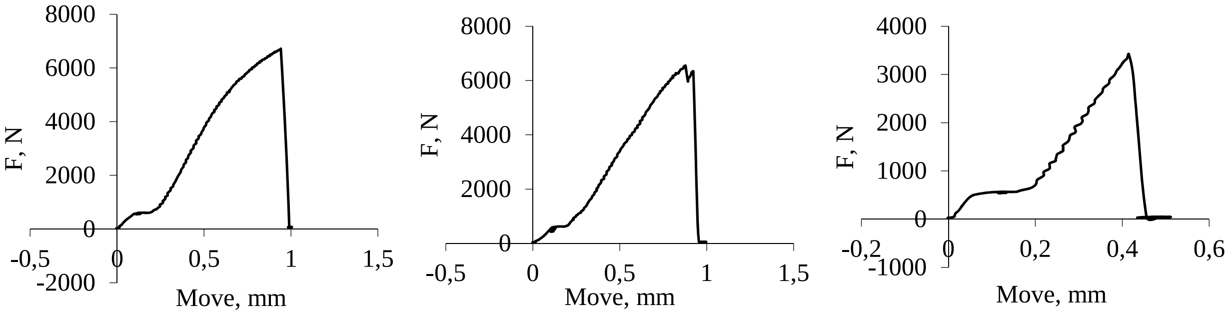
The next part of the study was related to the influence of the change of the crosslinker on the adhesion strength. The diagram of the force dependence on the move for crosslinkers GL2 and L are shown on Figures 2.a and 2.b, respectively. A greater elongation is seen in the case of crosslinker L. However, for bolide application, only the shear strength is important, and not the elongation, so the choice of the crosslinker should be made according to that criterion. Since the lowest value of the shear strength for the GL2 crosslinker is higher than the lowest value for the crosslinker L in further testing, crosslinker GL2 was used.



a) b)

Fig. 2. The influence of the change of crosslinker on the adhesion strength: a) crosslinker GL2, b) crosslinker L.

After the selected pre-treatment and crosslinker, the effect of the filler adding on the adhesion strength was tested. The addition of the filler affects the increase in viscosity, the adhesive has a consistency of the paste and it is very difficult to apply it in a uniform coating on the surface of the sheet. Glass and cotton fibers were used as fillers. Cotton fibers, unlike glass, are thin and elongated. This structure of cotton fibers makes it easier to penetrate the surface holes of the adherent, whose drawings are similar to morphology. Therefore, the addition of cotton fibers (Figure 3.b) resulted in more and glass (Figure 3.c) lower values of shear strength compared to the sample without filler (Figure 3.a).



a) b) c)

Fig. 3. The influence of the filler adding on the adhesion strength: a) without, b) cotton fibers, c) glass fibers.

Supercritical fluid processing of polymers: Current research and future remarks

Polymer processing was one of the earliest applications of supercritical fluid technology. Different processes involving supercritical fluids (SCF), such as polymer separation, polymer synthesis, formation of particles, fibers, foams and blends, impregnation of polymers, morphological modifications and polymorphic transformations, are widely investigated for use in pharmaceutical, food, and cosmetic industries, medicine, chemical industry, coatings industry. Using conventional techniques it is difficult to obtain an accurate control of material properties; moreover, these processes in some cases do not offer an environmental safe way of production. For these reasons, supercritical fluid (SCF) based processes are increasingly investigated for polymer processing. [8] The interaction of polymers with supercritical CO2 at elevated pressures is still under intensive research. Even if mathematical models can be used to predict the phase equilibrium data at unstudied conditions, parameters should be first determined experimentally.

Detailed investigations on the basic thermodynamical and transport data like phase equilibria, density, viscosity, dielectric constant and diffusion coefficient have to be carried out to obtain the data fundamental for the design of a process in order to fulfil consumer and economic requirements. Development and modification of the simple methods for determining these properties at the conditions, pertaining to the process is therefore an interesting topic of current research.

Conclusion

The following conclusions have been drawn:

- Pre-treatment of the surface of the sheet on which the adhesive is applied significantly influences the adhesion strength. Mechanical pre-treatment, surface grinding, changes the morphology and roughness of the surface, which leads to a better adhesion of the adhesive to the surface of the sheet and increasing the adhesion strength compared to the untreated sample. Mechanical pre-treatment in combination with chemical pre-treatment also has an impact on the change of morphology and contributes to improvement of the adhesion strength.
- The type of crosslinker has an effect on the adhesion strength.
- The filler additive adversely affects the properties of the adhesive joint due to the increased viscosity of the adhesive and its heavy application on the surface of the sheet.
- Parts of vehicle of Formula Student team "Road Arrow" are successfully bonded.

- The production of micro-composites using SCF techniques is still at the early stage of development; the researchers have done a large effort to obtain new materials and to demonstrate the potentiality of these processes.

Acknowledgment

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Supercritical epoxidation of grape seed oil

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1. Introduction

Polyurethanes (PUs) were discovered more than 80 years ago by Bayer et al. [1] and cover around 7.5% of European plastics converter demand [2], and are known to be a commodity polymer with a wide range of applications, such as coatings, adhesives, sealings and foams, which are the biggest of all, in terms of production and can be differenced in rigids, flexible or even viscoelastic foams [3].

PUs are defined as polymers that contain urethane groups (-NH-CO-O) in the main chain [4]. They are usually synthesized by the reaction of two monomers containing functional groups capable to form those urethanes, which traditionally have been a hydroxyl-bearing monomer (polyol) and a monomer with isocyanate groups [3].

Nevertheless, there are serious pollution problems in most of the conventional polyurethanes synthesis processes as well as their intermediates.

Both of the PUs common raw materials are petro-based, and therefore, non-renewable resources. As the polyol contributes to majority of the molecular weight, the idea to completely or partially replace it by a bio-renewable feedstock could be really interesting from an ecological approach. In this context, the utilization of plant oils is one of the most commendable alternatives. The ones that mostly consist on unsaturated triglycerides can form polymers through chemical modifications and so, they can substitute traditional petroleum resources and be precursors of valuable chemical products [5].

Castilla-La Mancha, a region located in central Spain, embraces one of the largest wine industry sectors in the world. As a result of its activity, approximately 100,000 tonnes of grape seeds are produced per year, with a content of oil near to 20 wt.% [6].

Its availability, relatively low price (as a consequence of being basically a by-product), high content in functionalizable unsaturated bonds, and the fact of being a sustainable resource make grapeseed oil an attractive choice as the backbone for the new chemical manufacturing.

Nowadays, PUs have a strong environmental and human health impact during the three steps of its life cycle. As isocyanates are being synthesized, phosgene, a lethal gas, is needed. Also, the most popular isocyanates compounds have been classified as CMR

(Carcinogenic, Mutagenic and Reprotoxic) by the REACH regulation [7, 8]. Finally, after usage, PUs are usually burnt and release isocyanate and HCN poisonous. Furthermore, the whole process covers an important ecological impact related to residues and emissions [9]. All these implications make it necessary to consider the development of a synthesis route that does not involve the use of isocyanates.

Non-isocyanate polyurethanes (NIPUs) currently arise as an option that solves all these inconveniences, and whose main route is based on the reaction between polycarbonates and polyfunctional amines to result in compounds known as polyhidroxyurethanes. They have the added advantage that polycarbonates can be obtained from renewable resources, such as the above-mentioned grapeseed oil, through consecutive reactions of epoxidation of the double bonds present in the triglycerides and oxirane ring opening by means of the introduction of carbon dioxide into the structure of the molecule.

With all this, the epoxidation of the oil becomes the first stage and that from which will derive the rest of processes that can lead to the obtaining of a clean polyurethane and respectful with the environment and the human health. In addition, an epoxy product can be used for multiple applications that differ from this [10], such as epoxy resins [11], painting [12], plasticizers [13] or thermosets [14].

Usually the processes that have been used to prepare epoxidized vegetable oils are based on the Prileschajew reaction which consists on the substitution of an unsaturation by an oxirane ring caused by a percarboxylic acid (peracetic or performic acids are the most common) that is formed in situ [15, 16]. Soluble mineral acids, commonly sulfuric acid, are used as catalysts for this reaction. Therefore, environmental concerns related to the handling of salts formed during the neutralization of the catalyst and technical and economic problems associated with corrosion and separation operations due to the use of both peracids and mineral acids, expose the need of a substitute for this technology [17].

Carbon dioxide reacts with H2O to form carbonic acid, and by analogy, when it finds hydrogen peroxide (H2O2), it is supposed form the corresponding peracid, named by some authors as peroxycarbonic acid [18]. This acid could replace traditional peracids as a cleaner and easier to handle alternative for the epoxidation of olefins in general and vegetable oils in particular.

Being so, not only CO2, but supercritical CO2 (scCO2) could be a great substitute for the common carboxylic acids used in Prileschajew reaction. Such claim is based on the properties of the supercritical fluids, with a density high enough to grant them a considering solvating power, also a lower viscosity and a molecular diffusivity significatively higher than the corresponding to the liquid phase, both of them related to the improvement of mass transfer. All these properties in addition to the non-explosiveness of the scCO2 make it a really attractive carrier in the epoxidation reaction process [19]. Finally, the use of the supercritical technology implies a main advantage consisting on the obtaining of a nearly pure epoxidized product without the need of neutralization procedure as it remains with a pH value close to 7, due to the ease separation of the scCO2 through a simple depressurization [20].

2. Experimental

Grapessed oil was kindly provided by Alvinesa S.A. The tetrabuthylammonium bromide (>98%), hexadecytrimethyl-ammonium bromide hydrogen (<98%), peroxide solution (30% wt), hydrogen peroxide-urea adduct (15-17% active oxygen basis) and sodium bicarbonate (99.5-100.5%) were supplied by Sigma-Aldrich. The tetrapropylammonium bromide (>98 %) was supplied by Fluka.

The experiments were carried out in a high pressure facility, shown in Figure 1, which is divided in 3 modules: Pressurization module, Reaction module and Depressurization Module.

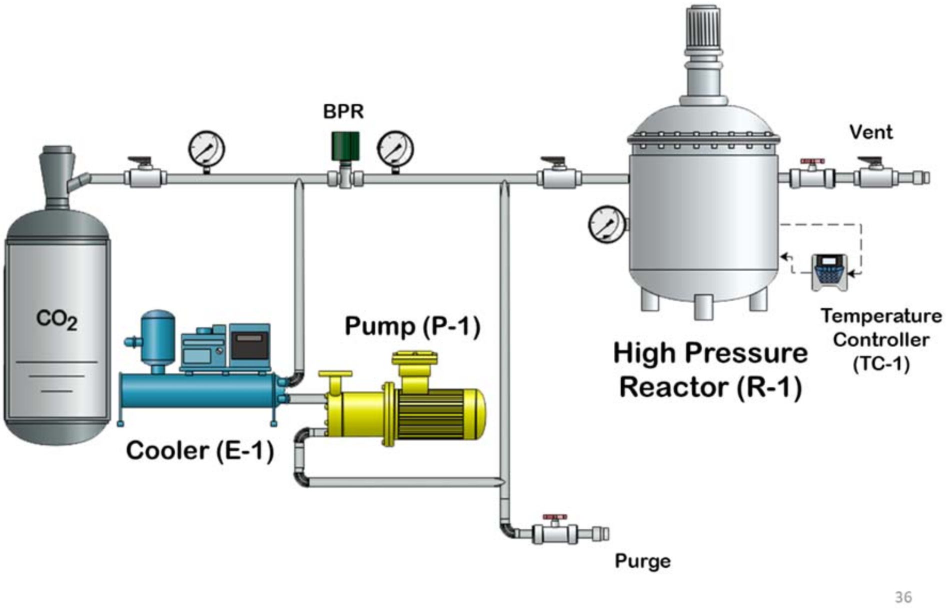

Figure 1. Experimental High Pressure Facility

3. Results and discussion

In this work, the supercritical epoxidation of grape seed oil is carried out at 150 bar, 40ºC during 15h. A preliminary study of direct influence of different additives (phase transfer catalysts or PTCs, $NaHCO_3$) was developed.

From these experiments (Figure 2), it can be derived the positive effect of sodium bicarbonate on the oxidant power of the generated peroxicarbonic acid, but mainly the PTC influence in the mass-transfer between phases (sc-CO_2-oil).

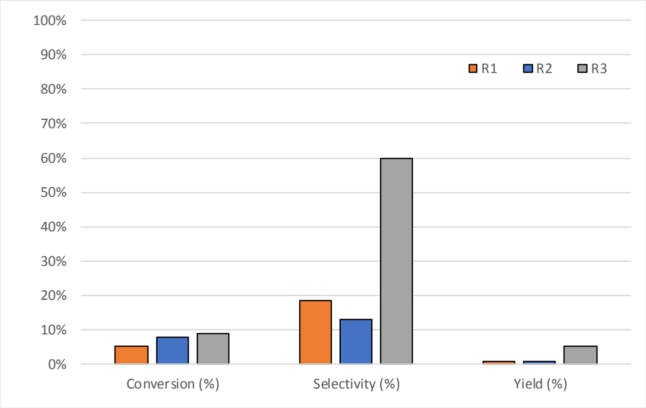

Figure 2. Analysis of oil sc-epoxidation performance with the addition of: R1: H_2O_2 (2)*; R2: H_2O_2, $NaHCO_3$ (2, 0.1)*; R3: H_2O_2, $NaHCO_3$, PTC (2, 0.1, 0.025)*
*molar ratio (referred to oil)

In order to optimize the epoxidation yield obtained in the product, different molar ratios between reactants were studied: H_2O_2 (2,6); $NaHCO_3$ (0,0.1,0.3); PTC (0,0.25,0.75).

The results obtained show some yields above 15%, even higher than those observed in the literature for supercritical epoxidation of soybean oil.

Once the reactants quantities have been fixed, the analysis of the pressure influence in the oxirane rings formation was carried out. From this study, it can be derived that a pressure increase in the scoped range (determined by facility limitations) has a positive influence in the overall epoxidation yield, that may be related to the enhanced solvent capacity of $scCO_2$.

Finally, a new perspective is explored, as the multiphase system is the main limiting factor to the performance of the epoxidation reaction because of the difficulty in the mass transference, a homogeneous reaction would be an interesting path to obtain a better product. With this in mind, the use of a non-aqueous H_2O_2 source is considered, being the compound formed by urea-hydrogen peroxide (UHP) the selected alternative to H_2O_2/H_2O solution. Through this approach yield and specially selectivity to oxirane formation is expected to be enhanced, as the hydrolysis of oxirane rings should be drastically reduced and so further oligomerization reactions, in addition to the aforementioned contact improvement between the main reagents.

4. Conclusions

The feasibility of supercritical epoxidation of grape seed oil is demonstrated.

The addition of NaHCO3 increases the oxidizing power of peroxycarbonate acid, resulting in increased conversion.

The use of a PTC improves the contact between phases, substantially affecting the conversion in a positive way.

In the studied pressure range, the reaction performance is positively influenced by the increase in pressure.

UHP is an auspicious substitute for aqueous H2O2 as a peroxidizing agent.

The reaction using UHP, at a pressure of 150 bar, results in a yield of 36.28%, currently the highest in a supercritical vegetable oil epoxidation.

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Enzymatic reactions in supercritical carbon dioxide

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Introduction

Human dietary fats and oils are mainly composed of triacylglycerols. Triacylglycerols are esters derived from glycerol and three long-chain carboxylic acids called fatty acids. According to the number of double bonds in their hydrocarbon chain, fatty acids can be classified into saturated, monounsaturated and polyunsaturated (PUFAs). PUFAs can be further classified based on their double bond positions into omega-9, omega-6 and omega-3 fatty acids. Omega-3 and omega-6 cannot be synthetized by human metabolism and are therefore called essential.

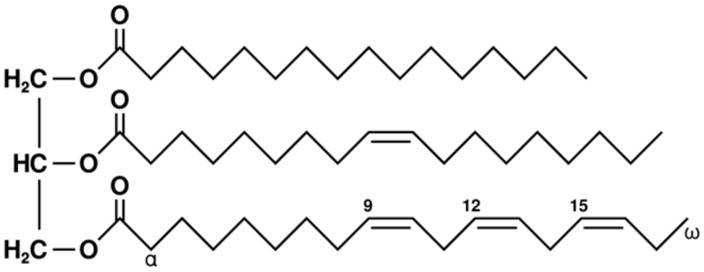

Fig. 1: Triacylglycerol

Consumption of high omega-3 and omega-6 oils has been recommended by worldwide healthcare authorities and many food supplements containing especially omega-3 fatty acids are available on the market. Not only is the sufficient amount of omega-3 and omega-6 fatty acids in human diet beneficial for health but also their ratio seems to play an important role[1]. However, only few sources of PUFAs are well balanced. In the western population the predominant source of PUFAs are vegetable oils high in omega-6 fatty acids. Complementary high omega-3 oils are mostly obtained from sea sources and are not abundant in western diet. Vegetable oils usually contain a few omega-3 fatty acids and therefore the ratio is shifted to the side of omega-6. Nevertheless, there are some vegetable oils like black currant seed oil or hempseed oil that contain considerable amount of omega-3 in the form of alpha-linolenic acid. Finding a method of refining these vegetable oils to obtain products higher in omega-3 fatty acids could be a way how to improve dietary composition for human population independent of sea resources.

One of the possible approaches how to obtain product enriched in omega-3 fatty acids is based on the fact that distribution of fatty acids in the triacylglycerols is not statistical, but some fatty acids are preferentially at *sn*-1 or *sn*-3 positions. Conversion controlled hydrolysis with *sn*-1,3 specific lipase would then produce free fatty acids with different fatty acid profile than the rest of the reaction mixture.

Using supercritical carbon dioxide ($scCO_2$) for this purpose seems to be advantageous since the mass transfer in $scCO_2$ is much faster than in the case of liquid solvents. It has been shown that commercially available immobilized *sn*-1,3 specific lipase Lipozyme is very stable under conditions of $scCO_2$ and it is able to maintain activity during hydrolysis reactions[2]. Supercritical carbon dioxide is generally well suited for food industry application considering its nontoxicity, inertness and overall environment friendliness. Supercritical CO_2 can be easily removed from reaction mixture by simple depressurization; also controlled depressurization can be used for subsequent separation of reaction mixture since various compounds have different solubility in the $scCO_2$.

Main objective of this project is to conduct *sn*-1,3 specific hydrolysis of selected vegetable oils with enzyme immobilized in packed bad reactor using water saturated $scCO_2$ as solvent and to optimize process parameters to yield mostly mixture of 1,2-diacylglycerols and free fatty acids. Differences of fatty acid profile between fraction of free fatty acids and fraction of diacylglycerols will be determined with HPLC-APCI. For better theoretical understanding of this process mathematical model will be proposed simultaneously.

Experimental

Scheme of the experimental apparatus for $scCO_2$ hydrolysis is shown in Figure 2. In the first step, $scCO_2$ is saturated with H_2O flowing through the column filled with demineralized water (2). In the next step, solvent passes through the second column (3) where the oil is dissolved. Resulting mixture flows to the reactor (4) with immobilized *sn*-1,3 specific lipase. Finally, the product is separated from the solvent via depressurization in expansion valve (5). In order to protect the enzyme from liquid water exposure and thereby its deactivation, the reactor is flushed with dry $scCO_2$ for several minutes after the reaction has finished.

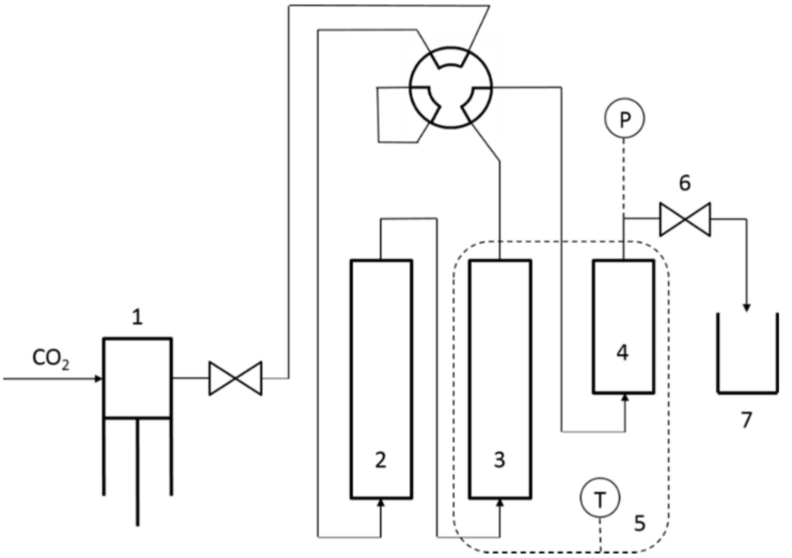

Fig. 2: Scheme of experimental apparatus: 1 – high pressure pump, 2 – column with demineralized water, 3 – column with oil, 4 – reactor, 5 – thermostat, 6 – expansion (micrometric) valve, 7 – glass vial

Summary

At this point, a few pilot experiments have been conducted and a method for fatty acid profile determination is being developed.

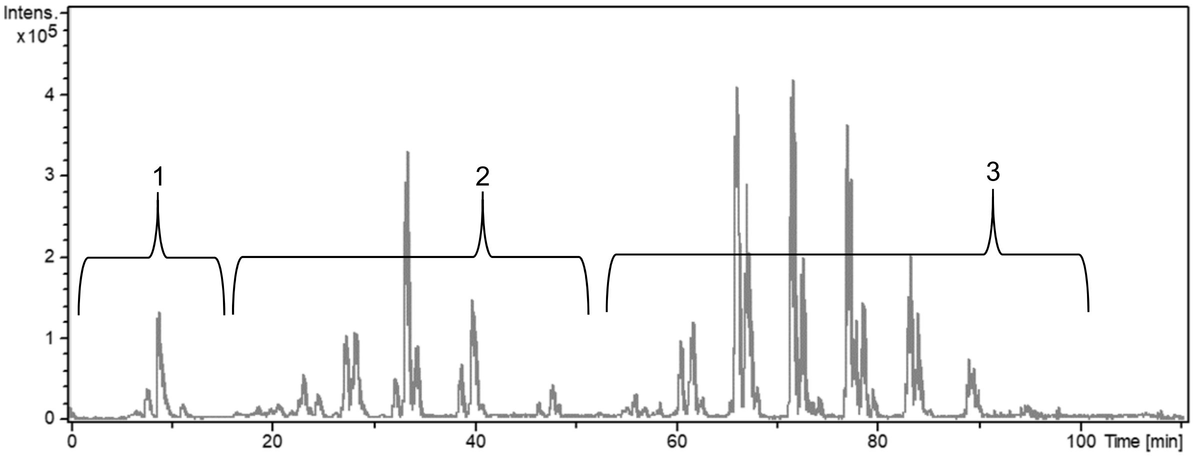

Fig. 3: HPLC chromatogram of hempseed oil hydrolysate: 1 – free fatty acids, 2 – diacylglycerols, 3 – triacylglycerols

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Pulverization of highly viscous polymer melts with a planetary roller extruder for selective laser sintering

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Introduction

The term additive manufacturing refers to different processes of joining materials in order to produce components layer by layer from 3D model data. Each additive manufacturing process is determined on the basis of the type of material (polymers, metals, ceramics), the state of the material (liquid, powder, threads), the principle applied (melting, curing, sintering) and the machine architecture [1]. As a result, various standard methods have been developed for additive manufacturing, including SLA (stereolithography), SLS (selective reader sintering), LMD (laser deposition welding) or FDM (fusion layering) [2].

The production of powders is still a major challenge for the SLS process. For the SLS process, the main focus is on the production of fine, spherical powder with narrow particle size distribution. In addition, different requirements are placed on the polymer powders depending on the area of application. The advantages and disadvantages of the previously powder production methods for the SLS process, such as cryogenic grinding, coextrusion and emulsion polymerization, do not allow any powder production under guarantee of the desired [2]. One of the possible manufacturing processes would therefore be the pulverization of polymers with a planetary roller extruder. The coupling of an extruder with a spraying process would function on the principle of a PGSS high-pressure spraying system (Particles from Gas Saturated Solutions), which enables the production of spherical, angular, fibrous or even porous particle shapes [3].

Experimental

The PGSS line consists of the following main elements: a single screw extruder (SSE), two gear pumps, a planetary roller extruder (PRE), a carbon dioxide station and a spray tower (ST). Figure 1 shows the diagram.

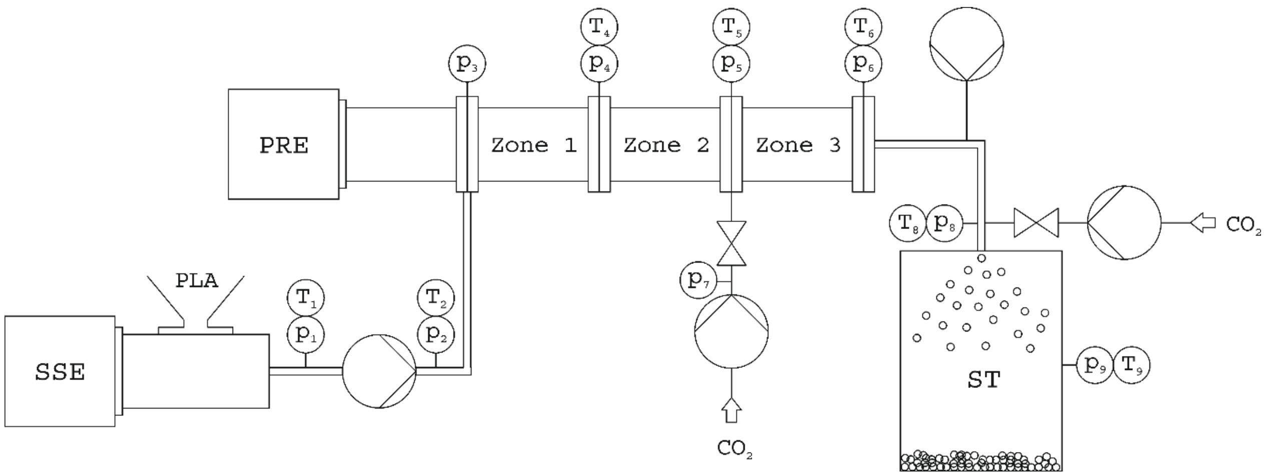

Fig. 1.: Schema of the plant

The single-screw extruder with a diameter of 45 mm consists of six heating zones which melt the polymer and prepare it for further processing. The melt is then conveyed by the first gear pump into the planetary roller extruder. The planetary roller extruder manufactured by Entex Rust&Mitschke has a central spindle with a diameter of 35.36 mm and rotating spindles with a diameter of 17.68 mm. The extruder is equipped with a central spindle with a diameter of 35.36 mm and a rotating spindle with a diameter of 17.68 mm. There are six transport and drying spindles (TT spindles) in zones 1 and 3 of the planetary roller extruder and six knob spindles in zone 2. Repeated rolling over a large surface area achieves a high mixing intensity and heat transfer. The arrangement is shown in Figure 2.

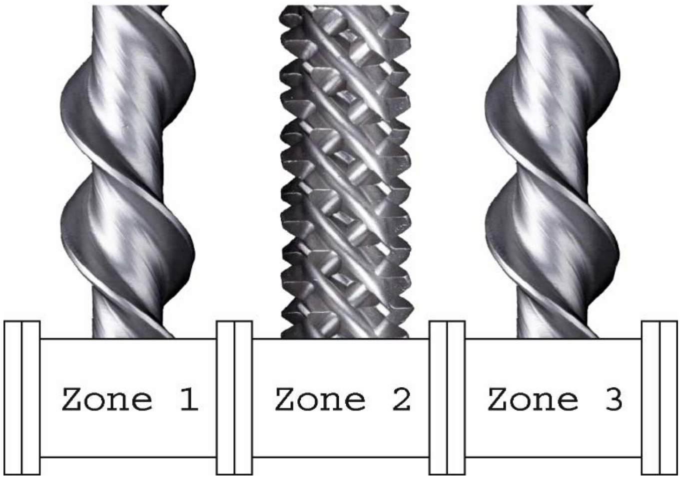

Fig. 2.: Planet spindle profile in the following zones [4]

The quality of the mixing is a result both of the setting of the pressures, temperatures and speed in the planetary roller extruder and of the properties of the polymer used and of the pressure and temperature of the carbon dioxide [5]. By dissolving compressed

CO_2 in the polymer melt, the viscosity of the mixture is reduced, which enables spraying [6].

Particles are generated by atomizing the melt as the pressurized melt expands through the nozzle into the spray tower. This results in a two-phase flow already within the nozzle due to the throttling. The characteristics depend, among other things, on the ratio of the length and the diameter of the nozzle [7]. The CO_2 escaping from the mixture behind the nozzle then cools the decomposing melt due to the Joule-Thomson effect. The pre- and post-expansion temperatures (T_9 and T_{10}) influence the transient heat transfer in the solidification process. The carbon dioxide content in the mixture is reduced from a maximum of 30% to 0.9-2.2% in the powder [3].

The powder produced for the SLS process must have many properties that are of great importance during sintering and determine the quality of the component [8]. One of the most important properties is the morphology of the powder. For proper flowability, spherical particles with a diameter of 45-90 µm are required [9]. The PGSS process is therefore particularly suitable for powder production for additive manufacturing.

Summary

Additive manufacturing is a technology that is currently gaining more and more importance. Despite the invention of stereolithography in the 1980s by Chuck W. Hull, there are still many obstacles that make it difficult to use addtivie manufacturing in industry [10]. One oft he main goals of this work is to manufacture matrials that have the desired properties. As a result, the pulverization of highly viscous polymer melts with a planetary roller extruder could become a possible future method for powder production.

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