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# Foreword

IProPBio – Integrated Process and Product Design for Sustainable Biorefineries, is a project funded within the Horizon 2020 Research and Innovation Staff Exchange programme, usually referred as "RISE".

The RISE programme has the main objective of cross-border and cross-sector collaboration through the exchange of staff among the participating entities. Our Consortium, composed by seven universities located in EU countries, one European small enterprise, one research center, three non-European Universities located in Mexico, United States, and Brazil, was gathered following the knowledge complementarity as lighthouse principle.

This principle appears to be the key of our success in targeting solutions to complex topics like the design of sustainable biorefineries, and implementation of innovative approaches to the valorization of biomass within the circular economy approach. The breakthrough results obtained will allow introducing cutting-edge management solutions and will encourage sustainability and competitiveness of EU in the long term.

Today, after almost two years of activities, we welcome you to the first IProPBio Workshop: Valuable products from residual biomasses. Towards a greener society (ProGreS): Biomass selection, characterization and valorization.

My deepest gratitude goes to all the partners that devoted time, resources and their passion in the achievement of the project objectives. The contributions in this Special Issue of the Bulgarian Chemical Communications present just some of the results realized opening our Institutions to our colleagues to create new views, to establish new collaborations, to face new challenges, to find new friends. Particular thanks are also due to Prof. Dragomir Yankov, whose efforts and perseverance made this Special Issue possible.

I would also dedicate separate thanks to partners from the third countries - Brazil, Mexico, and the States. Your devotion and exceptional hospitality are greatly appreciated.

As a Consortium, we are delighted to be hosted by one of the IProPBio lead beneficiaries - The Institute of Chemical Engineering, Bulgarian Academy of Science (BAS). BAS is the largest scientific organization in Bulgaria and this year it celebrates its 150th Jubilee.

During the First Workshop we will also have the chance to celebrate another jubilee, Sir William A. Wakeham, FREng. Sir William will turn half of the years of the Bulgarian Academy of Science and we all wish him many happy returns and years devoted to other inspiring works. His participation in the IProPBio Workshop is much appreciated as a priceless contribution and an opportunity for us all to benefit from his outstanding experience.

Finally, in my capacity as the IProPBio Coordinator I would like to assure you that it has been not only a pleasure but also a great honour to have the opportunity to get in touch with different cultures and so many brilliant minds.

Prof. Massimilano Errico
University of Southern Denmark
http://ipropbio.sdu.dk/

# *Keynote Lecture*

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# The dangerous nexus of process simulation, molecular modelling and physical reality

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The paper explores the dangers for careful process design using new materials and processes that may arise because of the state of understanding of the properties of those materials. Evolution of the sciences of molecular modelling and of thermophysical property theory is explored and it is clear that both have a considerable amount to contribute to careful process design in the future. However, it is equally argued that the evolution of the experimental database upon which the design of conventional processes has been based may not be evolving in such a favourable direction. By means of examples it is shown that what was the basis for the past success of thermophysical property provision, is being undermined by modern scientific behaviour and that there is ample evidence that when new materials are encountered the system that has been in place to validate data is simply lacking its full force.

The consequences of this lack of rigour in property provision for the databases on novel, perhaps especially biological processes, needs careful consideration.

**Key words**: Process simulation, thermophysical properties, transport properties, experimental technique, process design software

## INTRODUCTION

In the search for a more sustainable future for the world the chemicals and materials industries have sought to make their processes more efficient in terms of energy use, to reduce waste products and to use greener process routes to create products that are capable of re-use or recycling. In addition, there is a demand to move to feedstocks that are themselves renewable unlike traditional fossil feedstocks. The tools of the modern era of process-simulation have made it possible to conduct automated, intelligent searches of alternate process routes to products using these feedstocks [1]. At the same time the world of science has, in many areas, particularly in thermophysical property research moved away from the value attached to careful experimentation in favour of molecular modelling and cavalier experiments with excitement value! Such work is simpler, quicker and cheaper than careful experiments and leads to faster career enhancement!

At the same time, there is ample evidence that when inadequate comprehension of physical reality is combined with over enthusiastic simplification of either experimental design and implementation or automated design processes, gross errors of process design can occur even for conventional processes and materials. When the materials and/or processes are new or untried the likelihood of gross errors is exacerbated.

The development of the tools employed by the industry to design and optimise processes has been carried through by a combination of scientists and engineers with a relatively well-defined set of starting points and objective functions. A vast array of experience has been accumulated based upon processes of a conventional kind. As the simplest possible example Fig. 1 shows many of the elements of a conventional process that involve the familiar unit operations of mixing, heating, reaction, cooling and separation. Even if one does not consider alternative process routes to the same product the design of such a simple process requires a knowledge of the thermodynamic and transport properties of all the materials involved, the kinetics of the reactions and of the relationship of the design variables to those properties.

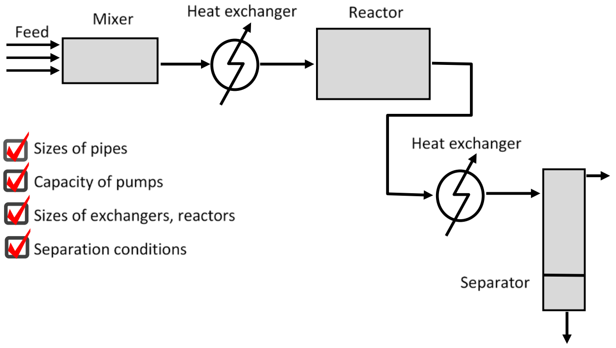

**Fig. 1.** Exemplar chemical process.

Let us take just one unit, a heat exchanger, illustrated in Fig. 2. Its design requires a specification of the needed duty, a selection of the type and then a deduction of the area of the exchanger based upon long-established empirical correlations of heat transfer for conventional

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materials and the thermophysical properties of the fluids involved. Both elements are now incorporated into design software that is available commercially and which has been thoroughly tested and validated. That this process of testing and validation is important is illustrated by the indication in the figure that costs accompany an over (or under) design of the exchanger. The costs can be expressed in financial terms for convenience but they can also be expressed in terms of environmental damage or waste [2]. The likely costs for an entire process are of course additive and depend upon the number of units.

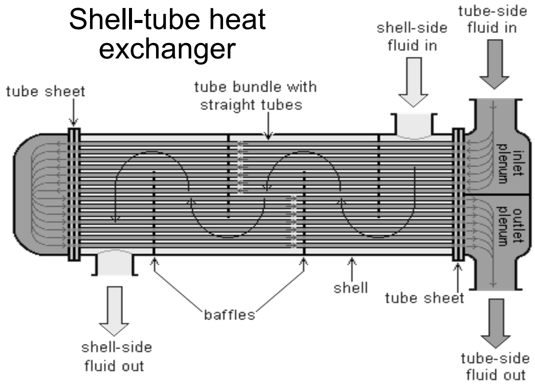

**Fig. 2.** Heat exchanger.

If some efficiency is attempted, as illustrated in the methanol synthesis reactor sketched in Fig. 3, then the problem potentially becomes more acute because here the heat exchanger is combined into the reactor so that any overdesign in the exchanger has consequences for the design and construction of the reactor as well [2].

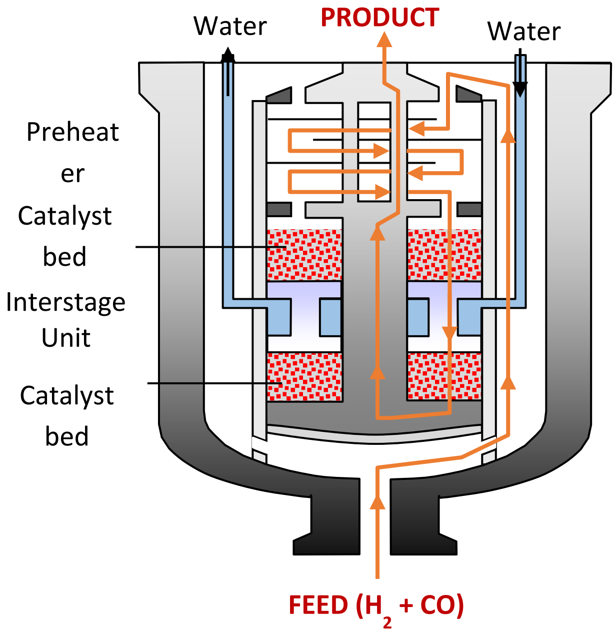

**Fig. 3.** Integrated methanol synthesis reactor.

In any realistic situation and perhaps especially if attempts are made to explore novel methodologies then there are many possible process routes to explore and need for confidence in the tools of design become ever more important. As an example, we consider Fig. 4 which depicts a solar capture system developed in Nevada, USA to generate electricity. USA DoE recently invested \$737 million into the Crescent Dunes Solar Energy Project in Nevada, which will generate energy well into the night by using molten salt as an energy storage medium. To do this the plant will focus nearly 20,000 heliostats upon a solar power tower filled with salt, heating the material to 840 K.

The use of a molten inorganic salt as the heat transfer and storage medium means that its thermophysical properties are important. As we shall see later the data on the properties of such molten salts are by no means well known and agreed even for the most familiar of them. It is the thesis of this paper that the tools of testing and validation that have been applied to date for these designs are now either under threat or falling into disuse and that this trend is dangerous. In order to make the argument we review the various parts of the validation process in turn and then point out why it may be less robust for new processes that employ different feedstocks and aim at different products.

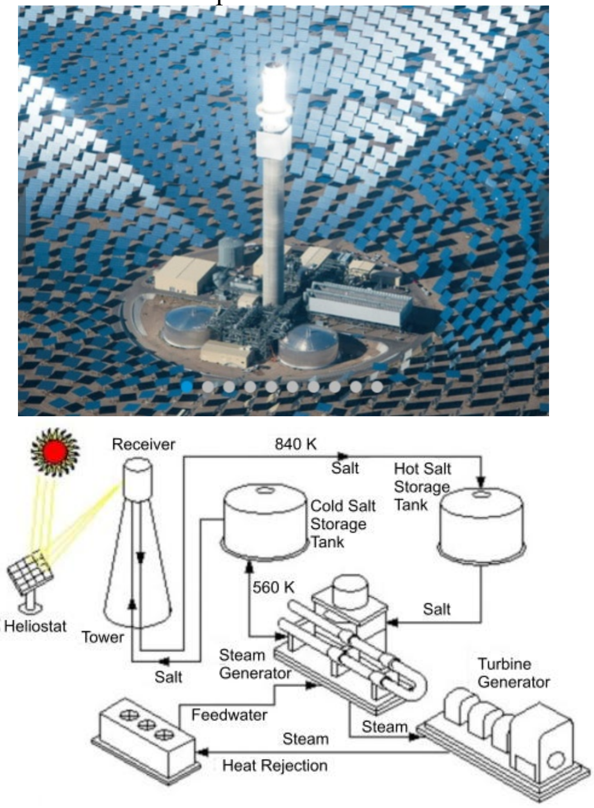

**Fig. 4.** Crescent Dunes Solar Energy Project in Nevada.

## VALIDATION PROCESSES

There are various elements of the validation process with which we are concerned here which is the provision of reliable thermophysical property data for the fluid systems under study. A similar argument might be advanced about the familiar correlations of the process industries, but we have sought to confine our consideration to the area we know best. They are:

- Molecular Theory.
- Molecular modelling
- Experimental measurements
- Internationally accepted data and correlations

### *Molecular Theory*

The only state of matter for which we have a rigorous, tractable analytic theory is the dilute gas. In that case we now have knowledge of the intermolecular pair potential for all the monatomic species and that allows the evaluation of all of their properties from a rigorous theory [3]. In the last few years it has become possible to extend this approach to polyatomic systems of very modest complexity such as carbon dioxide and water vapour but no further [4]. These developments have reached the point now, after about 100 years work, where the calculations are more reliable than experiment [4, 5], but it is important to recognise that was achieved in part by the existence of very careful experimental property measurements in the first place. Certainly, the dominant species of interest in the process industries and the states of matter of interest cannot be treated in this way.

Table 1 [47] contains experimental results, using the best possible experimental methods for both the viscosity and the thermal conductivity of the five monatomic gases at a temperature of 308.15 K and in the limit of zero density.

The measurements are independent of each other and absolute. The results are used to evaluate the Eucken Factor, *Eu,* defined by [3] as

$$Eu = \frac{2M\lambda_0}{3\eta_0 RF(T)} = 2.5\ (exactly) \qquad (1)$$

where the factor *F(T),* is an almost, model-independent factor from theory very close to unity and *R* is the Universal Gas constant.

Cencek *et al.* [6] developed the most accurate pair potential energy surface to date for Helium and used it to calculate properties of Helium. They recommended

$\lambda_{He}$ (298.15 K, 0.1 MPa) = (155.000 8 ± 0.001 5) mW m$^{-1}$ K$^{-1}$

Experimentally we find

$\lambda_{exp}$(298.15 K, 0.1 MPa) = (154.96 ± 0.30) mW m$^{-1}$ K$^{-1}$

These results and many other like them summarised in Refs [4, 7] demonstrate the mutual agreement of theory with the best experiments and, of course, provide mutual support for both. This is indeed very important when the same experimental methods are applied to other systems because the methods themselves can be said to be validated. That the same can now be done for small polyatomic systems is shown in Fig. 5 [5] where the deviations of the experimental results for the viscosity and thermal conductivity of methane are compared with theoretical calculations using a modern intermolecular potential for methane. The agreement is really excellent.

### *Molecular modelling*

In most cases of course this exact theoretical route is not available, either because the theory is not tractable (dense fluids) or because we lack intermolecular pair potentials for more complex systems. The approach of molecular modelling has been adopted to obviate these difficulties in one of two main ways.

The first, which was the only practical way forward until recently, was the adoption of simple molecular models of fluids that were tractable to approximate, or, occasionally, exact theory. Examples of this approach are various forms of corresponding states approaches to the prediction of the properties of fluids. The properties involved can be equilibrium or non-equilibrium and the systems involved can be single component fluids or mixtures.

The basic idea is founded upon the conformality of the pair potential for the interaction of molecules in the system so that all interactions in the reduced form

$$U/\varepsilon = f(r/\sigma) \qquad (2)$$

are identical. Here $\varepsilon$ is an energy scaling parameter and $\sigma$ a length scaling parameter.

**Table 1.** The Experimental values of the Eucken factors for the monatomic gases For reference see above [47]

| Gas | $\eta_o$ (μPa s) | $\lambda_o$ (mW m- K$^{-1}$) | $F(T)$ | Eu (Experimental) |
|---|---|---|---|---|
| He | 20.31 ± 0.02 | 158.4 ± 0.30 | 1.0042 | 2.494 ± 0.01 |
| Ne | 32.47 ± 0.03 | 50.41 ± 0.10 | 1.0035 | 2.503 ± 0.01 |
| Ar | 23.24 ± 0.02 | 18.18 ± 0.03 | 1.0012 | 2.503 ± 0.01 |
| Kr | 26.15 ± 0.03 | 9.722 ± 0.02 | 1.0006 | 2.497 ± 0.01 |
| Xe | 23.84 ± 0.02 | 5.656 ± 0.01 | 1.0001 | 2.497 ± 0.01 |

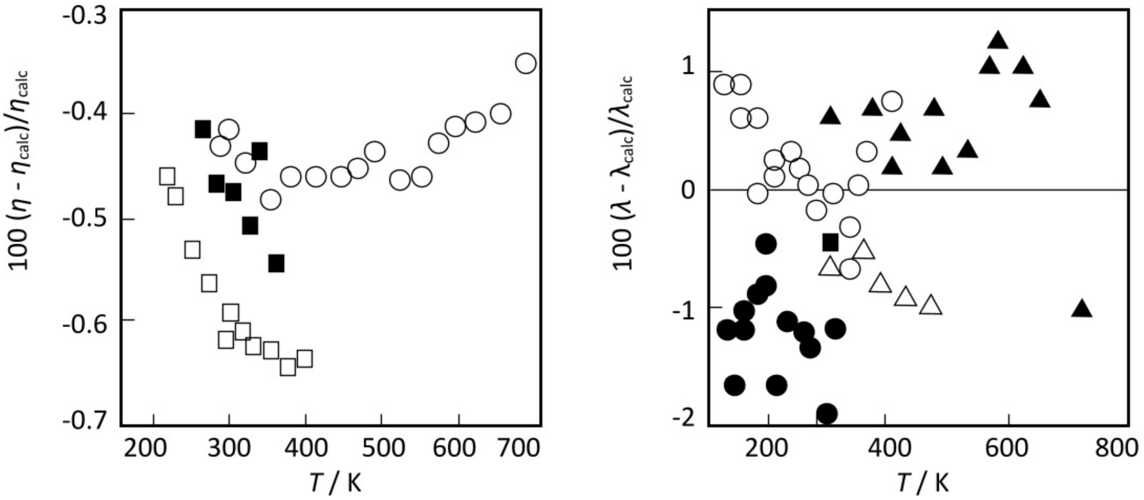

**Fig. 5.** Deviations of the experimental results for the viscosity and thermal conductivity of methane from theoretical calculations using a modern intermolecular potential for methane **[5]**

In the case of non-equilibrium properties it was possible to apply this idea to the representation of large numbers of pure and multicomponent mixtures of a wide range of spherical and indeed non-spherical molecules at low densities with a high degree of confidence supported by a large set of very accurate experimental data on viscosity, thermal conductivity and diffusion coefficients [8]. $U/\varepsilon$As just one example of hundreds Fig. 6 contains deviations of the measured viscosity of a number of multicomponent polyatomic gases from the predicted values.

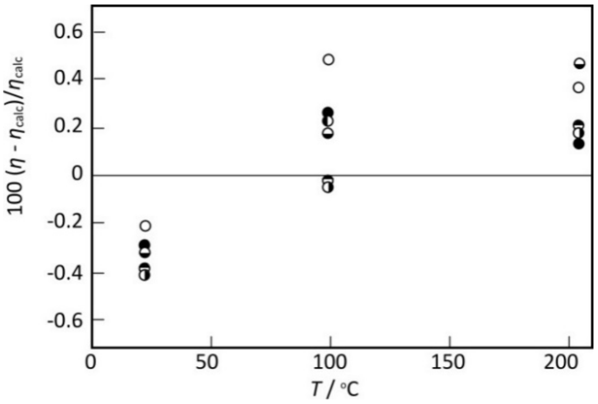

**Fig. 6.** Comparison between calculated and measured viscosity: ○ $CH_4$-$CO_2$-$CF_4$; ◐ $CO_2$-$CF_4$-$SF_6$; ◑ $CH_4$-$CF_4$-$SF_6$; ◒ $CH_4$-$CO_2$-$SF_6$; ● $CH_4$-$CO_2$-$CF_4$-$SF_6$ [9]

This approach gave a prediction capability for one property based upon measurements of another and over a wider range of temperature than the measurements could be performed.

Of course, the need is often for properties at elevated pressures or densities and, in this case, the dilute gas analysis was supplemented by a different molecular model for hard-spheres where the approximate Enskog theory of dense fluids [10, 11] could be applied to describe the density dependence. This allowed the development by Vesovic and Wakeham [11, 12] of methods for predicting the properties of dense gas mixtures using only the properties of the pure gases and some results from rigid sphere theory. Again, these methods have been validated for predictions using a wide range of accurate experimental data on well characterised fluid systems [12]. That gives confidence in the application of the procedures for the prediction of data on systems where there have been no measurements. As an example of what can be achieved using this VW method and experimental data for pure fluids only, Fig. 7 shows the deviations between the predictions of the revised VW scheme [11] and the measurements of Schley *et al.* [13] for natural gas containing only traces of higher hydrocarbons. The scheme performance deteriorates somewhat if the same model is used for heavier long chain hydrocarbons although recent improvements have been made [12].

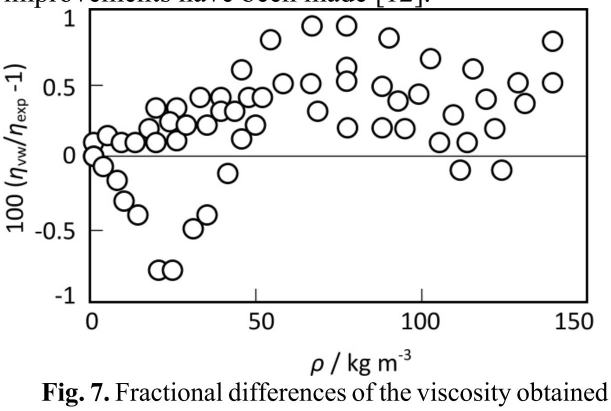

**Fig. 7.** Fractional differences of the viscosity obtained using VW-sphere, $\eta_{vw}$, from the experimental data for natural gas of Schley et al. [13], $\eta_{exp}$, as a function of the density $\rho$.

Dymond and Assael [14] have made use of a slightly different form of the Enskog theory to develop a methodology for representing and predicting the transport properties of dense liquid systems, particularly hydrocarbons. There have been several extensions of the Dymond and Assael scheme to higher densities [14].

There are, of course, equivalent approaches for equilibrium properties including phase equilibrium for well characterised systems simple molecular models, such as that which leads to the Van der Waals equation and its derivatives, and have achieved considerable success. Perturbation theories have also enjoyed considerable success

One of the most successful modern schemes for predictions and representations for more complex fluids is based upon the SAFT equation of state which, again, exploits simple molecular models for those systems. This has recently found application in transport properties as well as equilibrium properties [15]

There are numerous other examples of prediction methods for properties based upon different molecular models and the state of the art is summarised in Refs [11, 14-16].

A second strand of molecular modelling is more recent and exploits modern computing power. Using moderately realistic potential models for two or more body interactions it is now possible, via molecular dynamics simulations or Monte-Carlo-equilibrium simulations to calculate the transport properties or equilibrium properties of a modest ensemble of molecules that can be argued to be representative of the real fluid. This approach has developed rapidly, and quite complicated systems can now be studied. In that context water in the condensed state is a particularly interesting material because of its complex structure driven by hydrogen bonding. Although early calculations using proposed potential models were only able to predict the transport properties of water qualitatively, the most recent attempts, exemplified by the use of a potential from 2005 [17, 18] achieve quantitative agreement. The results are somewhat better for other hydrogen-bonding liquids such as alcohols where deviations of about 20% are found. Fig. 8 contains a comparison of the results of simulation for water with experiment. The attempts to perform such calculations have progressed much more rapidly than the experiments of high accuracy on such systems. They are now beginning to be simulations of complex systems such as might be involved in new green and biological processes.

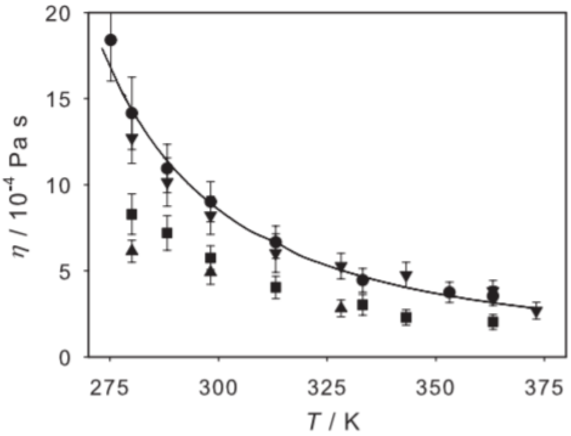

**Fig. 8.** Temperature dependence of the predicted shear viscosity of pure liquid water at 0.1 MPa.

### *Experimental Measurements*

We wish to concentrate here on transport properties which are our own main interest but we must say few words about equilibrium properties. The measurements of the equilibrium properties of fluid systems have been carried out for several hundred years, in the case of the density for example, because it was possible to conduct measurements of this kind with very simple equipment. For example, even the simplest form of specific gravity bottle enabled liquid densities to be measured. The phase behaviour of simple two component often two-phase systems could also be measured by visual observation and this formed the initial sort of data used for testing and validation of equations of state. Slowly the measurement techniques became ever more precise and with that followed the refinement of equations of state capable of representing all the equilibrium properties of pure fluids. An example is the IUPAC Equation of State for Ethylene [19]. The equations were often able to reveal inconsistencies in experimental data for different properties by virtue of the precision of some data and were thus able to highlight experiments that were in error. There are now commercial companies manufacturing devices for very high precision measurements of equilibrium properties of fluids such as the vibrating-tube densimeter for liquids [20, 21], the Rubotherm magnetic balance devices for gas and gas mixture density [21, 22] and the differential scanning calorimeter for heat capacities [21, 23]. It remains true that the quality of the results obtained with these devices depends upon the users and their understanding of the method. However, that has not prevented hundreds of papers being published in the scientific literature every year reporting, for example, excess volumes of binary and ternary mixtures of liquids over a very limited temperature range around ambient. The papers usually make entirely hand-waving deductions about the intermolecular forces and the sign of the excess volume. The next paper from the same laboratory then does the same thing for another pair of liquids from bottles on the laboratory shelf. This habit has earned the name of such work 'stamp-collecting' because it has neither scientific utility nor engineering value since the systems studied are usually simple ones rather than useful ones!

It is interesting that not until the 1970's did precise and accurate means of measuring the transport properties of fluids became available [24, 25]. The probable reason was that all such properties involve a dynamic process in a non-equilibrium state and in most cases it is very difficult in a fluid to isolate one dynamic process free from all others so that a mathematical model of the experimental process can be developed for analysis of an experiment. The most obvious example is the fact that, on earth, natural convection and radiation accompany the process of thermal conduction in any fluid within a temperature gradient where that gradient is not everywhere aligned with and opposite to the gravitation acceleration. Another important reason was that for many years the measurements had been the preserve of chemists and they tended to work in glass with little regard for mathematical analysis of the often complicated, processes involved in the measurement. Improved comprehension of all these features and the involvement of engineers and physicists in the field have changed the approach of careful experimenters markedly. However, this has not been a universal phenomenon.

The essential features of a good measurement of a physical property, whether equilibrium or not are:

1. A recognition that a thermophysical property, such as thermal conductivity, is only really defined for a single-phase material in the bulk.
   - materials may be anisotropic but must be otherwise homogeneous.
   - only using this definition is it possible to assert, without ambiguity, that the property is a function of the thermodynamic state variables $(P,V,T,x)$ alone.
2. It is perfectly legitimate in engineering terms to discuss the properties of multiphase materials and inhomogeneous materials as 'effective' or 'apparent' properties.
   - but the results obtained for the effective property could depend upon the method of measurement or the size of the sample employed.
   - the size of the sample may also be an issue when surface effects can dominate bulk effects such as in very thin films.
3. The measurement should involve a minimal disturbance of an equilibrium state.
   - therefore, we use small departures from equilibrium, so that the accompanying equilibrium state can easily be defined.
   - high sensitivity of measured variables to quantity being measured is essential.
4. A theoretical working equation or a numerical solution of sufficient accuracy for the governing differential equations that describe the experiment.
5. A practical realisation of the experiment that has small departures from the theoretical model is essential.
6. Each of the departures from the ideal model can be analysed to introduce small corrections to the working equation that can be quantitatively evaluated through theory and applied to the results.
7. It should be possible to demonstrate that the theory of the experiment exactly describes what is observed.
8. If possible conduct absolute measurements in which all parameters of the instrument are determined independently and by means traceable to fundamental units or constants.
9. If an absolute measurement is not possible, calibration to determine one parameter of the instrument with the aid of a standard reference material is possible.
   - The parameter determined should agree with direct evaluations of the same quantity from theory within its estimated uncertainty.

It has proven possible to satisfy all these conditions in some laboratories with respect to the properties of fluids. We would single out the laboratories of

- Joseph Kestin in Brown University, USA, for viscosity and thermal conductivity over a wide range of conditions,
- Mike Moldover and his colleagues in NIST, Gaithersburg over many years beginning in the 1930's,
- Akira Nagashima in Keiuo University, Japan,
- Marc Assael, from Aristotle University in Thessaloniki,
- Peter Dunlop in University of Adeleid Australia,
- Eckhard Vogel in Rostock University and Alfred Leipertz in Erlangen University and their colleagues in Germany, and
- Harald Oye in Norwegian University of Technology in Norway.

However, it has never been the norm to perform such careful experiments and we now see the dangerous signs of a complete disintegration of the protocols that stem from the principles set out above. We shall provide some illustrations of the good and then rather more examples of the bad extending over almost a century and it will become clear that the situation is deteriorating for reasons upon which we shall speculate.

### *Some Good Practice*

We choose three examples only which illustrate good practice.

The first is the capillary viscometry carried out in NIST where, over some 25 years, across World War II an instrument was developed to make absolute measurements of the viscosity of liquid water at 20 ◦C [26]. The resulting value of the viscosity of water is still the standard value adopted universally today. More recently NIST have used capillary viscosity in gases in conjunction with the best possible pair potentials for the interactions among monatomic gases to make exceedingly precise measurements [7].

Those values have supplemented those made with our second example, the oscillating disk viscometer employed in USA, Japan and Germany to provide standard reference values for the viscosity of gases. The concept of the torsional pendulum comprising a flat disk suspended in the fluid originates with Maxwell but Kestin and his colleagues [27] employed careful fluid mechanical analysis of the complex flow pattern around such a disk to design and then build instruments where it was possible to make absolute measurements of viscosity over wide ranges of conditions These devices also took several decades of work to achieve their result with the work of Vogel in Germany the most recent [28] We have referred to the validation of these results earlier.

The third is the transient hot-wire technique for the measurement of the thermal conductivity of fluids. It is in essence a very simple technique in which a very thin metallic wire is heated by ohmic dissipation when immersed in the fluid. It is possible to analyse this technique almost exactly mathematically, initially analytically and now numerically so that there exists an exact theory of the method. In its current form it was pioneered by Haarman [29, 30] and made possible by modern electronics because the entire heating process can be limited to a duration of one second during which time convection has a negligible effect on the heat transfer. It is also possible to confirm that the method operates in accord with the theory of it, as Fig. 9 illustrates. Here we compare the predicted behaviour of the temperature of the heated wire with the observations in one fluid.

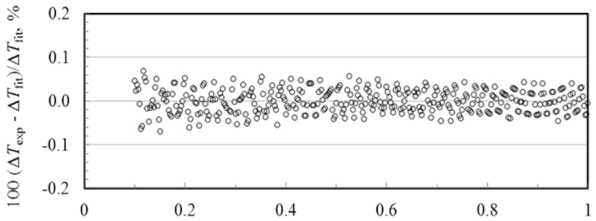

**Fig. 9.** Differences between the experimental temperature rise of the hot wire in water from the theoretical values, as a function of time.

The deviations are random and exceedingly small. These instruments employed in the laboratories of the authors [31, 32], Nieto de Castro *et al.* [33] and Perkins *et al.* [34] are responsible for most of the accurate thermal conductivity data for fluids.

### *Some Bad Practice*

We provide here rather more examples of bad practice in measurement than of good because, that is the balance in the field at present and because we wish to make the point that modern measurements are not to be trusted unless the full pedigree of the method is exposed and tested by researchers. In part, this is because of the growth of the popularity of commercial instruments for measurement and the fact that modern research (or possibly researchers) cannot spend decades to perfect a technique. Readers should note that these comments apply to thermodynamic and transport property measurements and not, for example, to measurements of time or length or voltage where commercial instruments have long been validated against agreed and accurate standards.

We begin with what is called variously the Transient Hot-Wire probe or the Transient Needle Probe. It is illustrated schematically in Fig. 10, and it consists of a wire and a resistance thermometer embedded within a solid powder itself held within a stainless steel sheath. The entire probe is immersed in the fluid of interest and the wire heated electrically while ‘its’ temperature rise is measured with the thermometer. The word ‘its’ is in quotation marks because it is not clear that the thermometer is measuring anything but its own temperature.

The theory applied to this device to deduce the thermal conductivity of the fluid is exactly that which we illustrated above for a very thin wire immersed directly in the fluid despite the obvious differences. The manufacturers themselves, who designed the device for on-site measurements of the thermal conductivity of rocks and concrete in a civil engineering context, seem to be aware of this problem as exemplified by their literature. Researchers looking for rapid results and instant fame are not it seems!

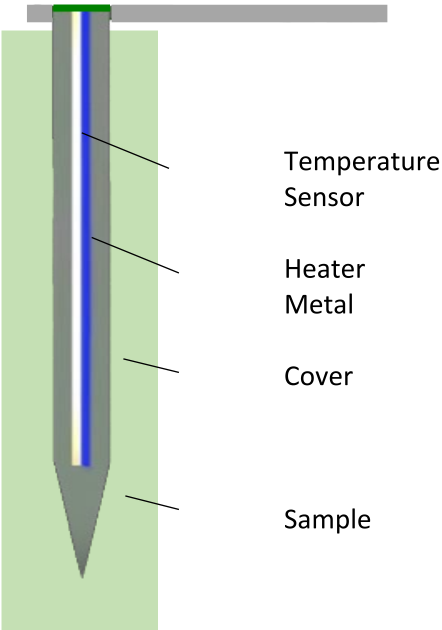

**Fig. 10.** Transient Hot-Wire probe.

A second device which has proved very popular is the transient hot disk, developed originally for solids. Its sensor is illustrated in Fig. 11 and its working principle is that an electrical current flows in the spiral that comprises the disk and heats the spiral, at a rate determined in part by the thermal diffusivity of the surrounding fluid. Unfortunately commercial versions of this device do not make use of the full theory of the instrument, which is mostly available, but they simplify it. In addition, the timescale of measurements in fluids are such that convective heat transfer is inevitable although its presence is universally ignored and no tests for its presence are applied.

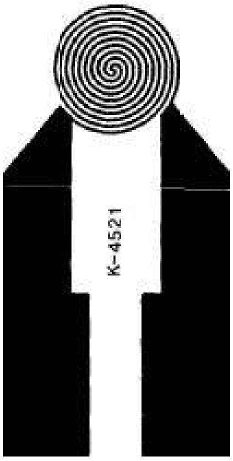

**Fig. 11.** Transient hot disc.

Thirdly, a technique known as the 3-omega technique was developed for solids where it is very successful for certain classes of material and is extremely elegant. It is illustrated in Fig. 12. A wire in contact with the test material is heated with an alternating current at a frequency ω. As a result there is, of course, a voltage change across the wire which oscillates at a frequency 3ω. The rate of evolution of that harmonic voltage change, which can be monitored by modern lock-in amplifiers can be related to the thermal diffusivity of the material. This technique, which is operated in essentially a continuous mode, has also been applied to fluids. It seems not to have occurred to its users that as well as the component of the voltage oscillating at 3ω there are other components, in particular a unidirectional temperature increase which, of course, drives convection for which no account is taken.

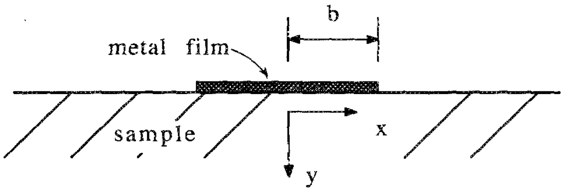

**Fig. 12.** The 3-ω technique.

The fourth example is from capillary viscometry, which is, perhaps, the oldest technique for measuring a transport property. The Montreal Protocol on the phasing out of certain refrigerants in favour of those less damaging to the Ozone layer in 1987 stimulated an intense fashion in conducting measurements of the properties of the proposed replacements. This fashion led to an enormous increase in the number of people interested in property measurement. Indeed, the measurement of the properties was sorely needed. However, among those attracted to the study were many with no previous experience of the field. They rushed in where angels had feared to tread and set out with simplistic equipment and theories of it to be the first to conduct measurements on the new systems. One such technique adopted was the sealed capillary viscometer illustrated schematically in Fig. 13 which was applied to the measurement of the viscosity of 1,1,1,2-tetrafluoromethane (R134A) very early on in the study of the fluid.

An example from diffusion of bad practice is the development of the Taylor dispersion technique for diffusivity measurements in liquids [36]. In this technique a small pulse of one fluid is injected into a stream of a second fluid flowing in a long circular section tube. The combined action of the laminar velocity profile in the tube and radial diffusion lead to an eluted pulse of the second material downstream from the point of injection whose width is a measure of the mutual diffusion coefficient of the two species. In order to make the measurement possible it is necessary to use a capillary tube a few mm in diameter and some 30 meters long. To make the system practical it is coiled so as to be able to immerse it in a thermostatic enclosure. Unfortunately, early proponents of this system did not realise that when the capillary tube is coiled a new velocity component is generated in the flow in the tube which affects mixing. The effect is large unless the Reynolds number is small and the coil radius large so the neglect led to large errors in early work before the full theory was evaluated.

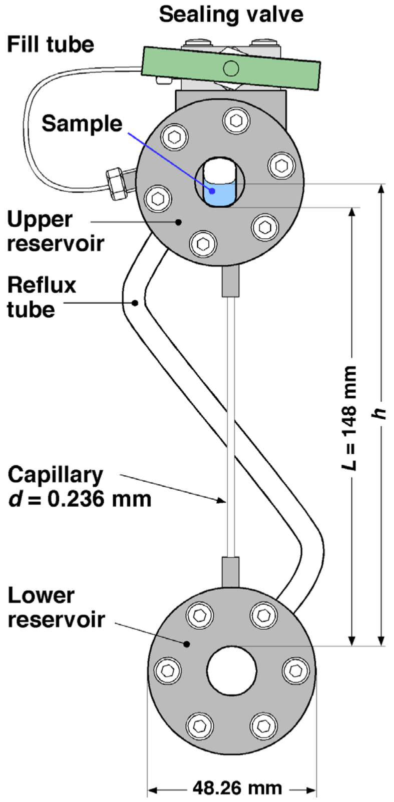

**Fig. 13.** Sealed gravitational capillary viscometer with straight vertical capillary developed at NIST [35].

Finally, returning to thermal conductivity many authors have been attracted by the idea of using a very much shorter hot-wire system, illustrated in Fig. 14, with the same theory as we set out earlier essentially for an infinitely long wire.

They seemed to forget that in such a case the longitudinal heat conduction to the supports of the wire can dominate radial conduction through the medium under test so that nowhere does the wire attain the temperature of an infinite wire so that the entire measurement is invalid. This has not prevented its use and papers being published! More recently a few authors [37, 38] have implemented computer solutions of the full heat conduction problem including the axial conduction in solids although it remains unclear what particular advantages this device holds over the more conventional system.

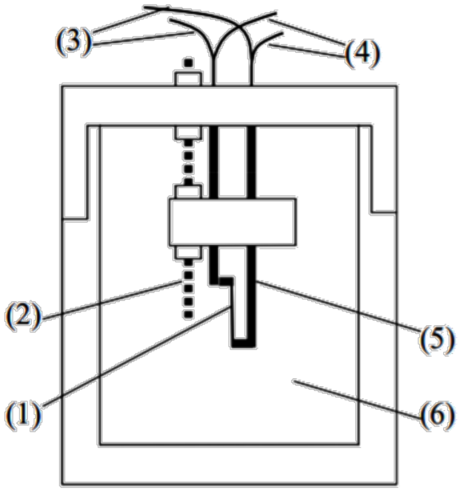

**Fig. 14.** Short transient hot wire (10 mm length, 50 μm radius). (1) Hot wire; (2) thermocouple; (3) voltage leads; (4) current leads; (5) Pt holder; (6) vessel.

### *The results*

This catalogue of failures in proper experimental protocol of course leads to varying degrees of chaos in the field as we shall illustrate here. First, let us look at the simplest system of all perhaps, which is toluene. Its thermal conductivity has been measured for almost a century and the value appears to have been decreasing in time as Fig. 15 shows.

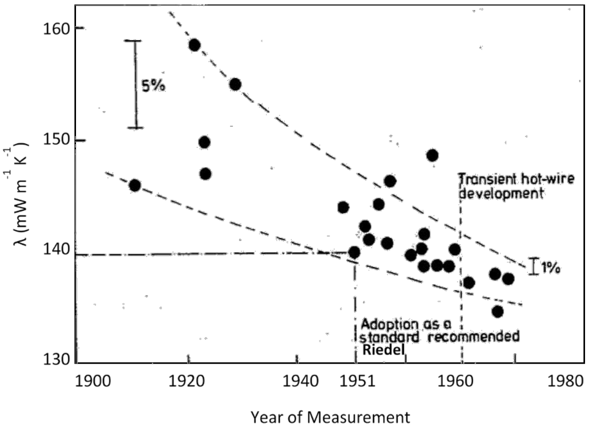

**Fig. 15.** Reported thermal conductivity of toluene as a function of the year of measurement.

The decrease was slowed when a standard recommendation was made, possibly because it was thought the ‘right answer’ was known! This illustrates the tendency to conform among researchers to an accepted result, which is one obvious danger.

In Fig. 16 we plot the deviations of two sets of early viscosity measurements for R134A from the latest validated values. These measurements were conducted in coiled and sealed capillary systems and it is clear that the measurements were up to 35% in error because of a neglect of these two effects.

If we consider less straightforward systems such as the molten metals, Fig. 17 illustrates the variation that can be found between the results of various measurements reported by a variety of techniques over time. A similar situation persists for molten salts as Fig. 18 exemplifies.

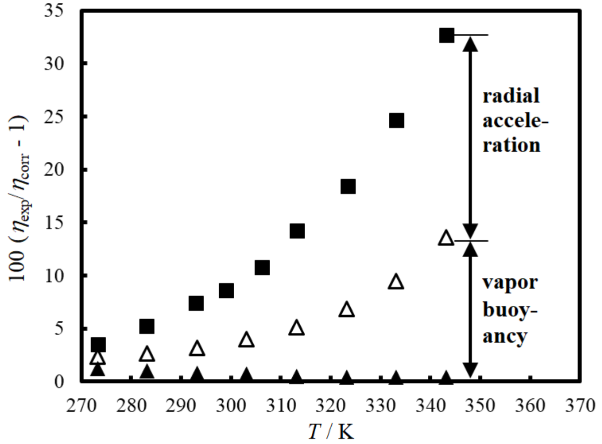

**Fig. 16**. Fractional deviations of the measured viscosity, for 1,1,1,2-tetrafluoroethane (R134a) from the accepted value, as a function of temperature.

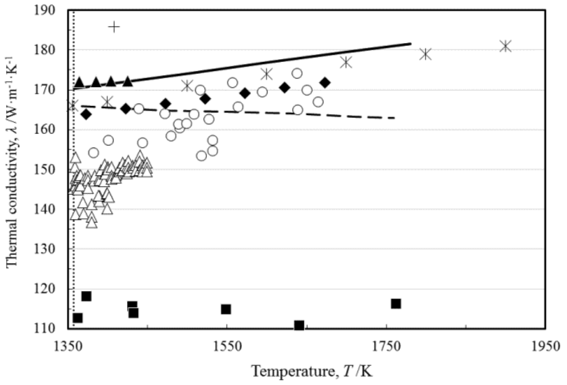

**Fig. 17.** Measurements of the thermal conductivity of molten copper.

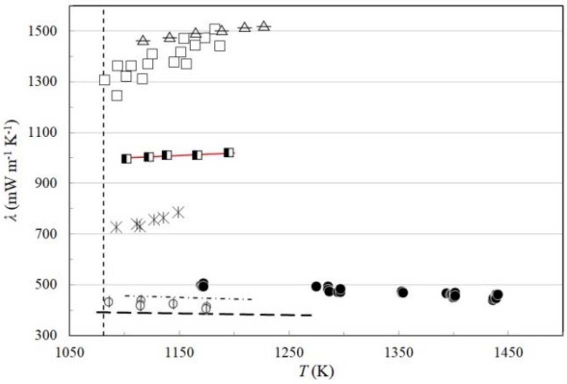

**Fig. 18.** Measurements of the thermal conductivity of molten NaCl.

It may be thought that this is all in the past but we shall now show you that the situation persists in the 21<sup>st</sup> Century. The relevant world became very excited in 1995 when it was claimed by Choi *et al.* [39] that the thermal conductivity of a base coolant such as water could be improved by 300% by adding a few volume percent of nanoparticles of alumina. The implications of this for heat transfer systems of all kinds were obvious and it stimulated an enormous stream of papers on the subject. Some showed the same effect, others no effect; some espoused theories to explain the phenomenon and there were computer simulations purporting to explain it. When you review all the evidence dispassionately a depressing picture emerges as illustrated in Fig. 19.

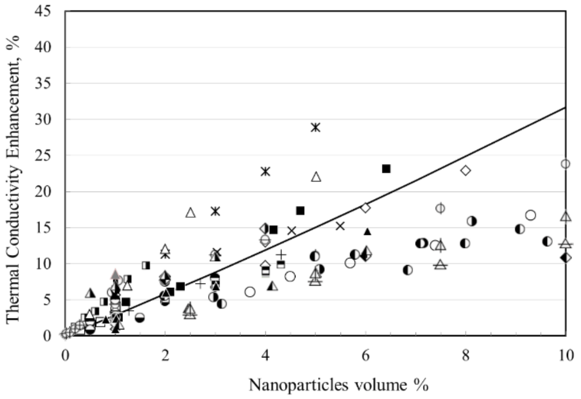

**Fig. 19.** Measurement of the enhancement of the thermal conductivity of $H_2O$ when alumina nanoparticles are added.

This plot shows the enhancement of the thermal conductivity of the base fluid by the addition of nano particles for an $H_2O+Al_2O_3$ system as measured by a wide range of authors and techniques; most of which we have described. It is clear that there is no consensus. We note, in the context of earlier observations, that many of the authors of these papers have no previous record of having been engaged in the measurement of the thermal conductivity of any homogenous materials.

However, if we eliminate from consideration the techniques that do not have a proper theory or were not applied properly, the picture becomes clearer (Fig. 20) and we see that there is a modest increase in the thermal conductivity which is very close to that expected from a simple theory originating from Maxwell [40]. Indeed, the increase is usually so modest that any heat transfer benefits are usually offset by the concomitant increase in the viscosity of the system.

The amount of money, energy and scientific effort devoted to this situation, merely driven by an experimental error, is frightening. The reader will, of course, note the role of fashion and excitement in this example!

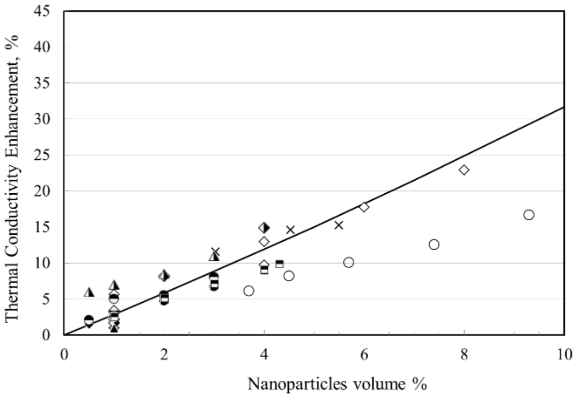

**Fig. 20.** Selected measurements of the enhancement of the thermal conductivity of $H_2O$ when alumina nanoparticles are added.

### *Internationally accepted data and correlations*

Driven, in part by this failure of past experimental studies of properties to be informed by careful, theoretically-sound methodology, the international community has relied upon an alternative method to determine validated data for the transport and equilibrium properties of fluids.

The effort was initiated by the International Association for the Properties of Steam (IAPS-Now IAPWS) in the 1920's. As its name suggests it was set up to produce internationally agreed values for the properties of Water and Steam largely for the steam boiler and turbine industries. Its initial product will be familiar to those of a certain age as the International Steam Tables [41]. These replaced, or were the source of, National Steam Tables used by engineers for all designs involving water and steam, and their international pedigree ensured transportability of designs across international borders replacing the disputes about the properties of Steam that were then extant. The work took many years and transcended hot and cold wars, and involved all countries of the world including those at war!

The effort continues today although the product is now a set of equations to describe all the properties of water, ice and steam as well as of heavy water [42]. The equations are available inside most respectable design software.

The basic approach of the international task group was to examine critically every measurement made of the properties of water and steam, to select the best measurements using the criteria we have outlined earlier, to reject all others or give them a small weight, to use theory where appropriate and then to produce a set of properties or an equation best representing the acceptable data. Of course, this process stimulated many more good measurements as well as generating enmity when particular measurements were deemed unacceptable.

This same methodology was adopted by two subcommittees of the Thermodynamics Commission of the International Union of Pure and Applied Chemistry (IUPAC). One, for thermodynamic properties, established in the 1950's a small group in London devoted to the production of books of the tables of the thermodynamic properties of important single substances. An example is the text on ethylene [19] which is a substance traded internationally in large quantities. For the process of custody transfer it was helpful (to say the least) if both sides of the border agreed on the density of ethylene at the transfer point because what was transferred was measured in volume but paid for by mass!

The second subcommittee, for transport properties (now the International Association for Transport Properties (IATP)) [43] had an obvious focus and is still operating today. It has produced many representations of the transport properties of fluids from the simplest low density gases to organic liquids to molten metals and molten inorganic salts, as well as three books [25, 44, 45]. Again, this work is endorsed internationally and is incorporated into modern design software. Work of a similar kind is conducted still in NIST, in USA and promoted through the Journal of Physical and Chemical Reference Data [46] but on a much smaller scale than hitherto and without international endorsement.

### *The future for processes including biological and novel other materials*

The near universal adoption of computer-based design software means that the properties of the materials embedded in this software often have an unknown pedigree. In what we have said above it is clear that for familiar and important materials encountered in the process industries to date there are many reasons to have faith in the numbers embedded in the codes. However, we have also shown that whenever materials are new and the topic becomes hot or fashionable, there is a rush to experimental measurements of properties by researchers ill-equipped for the task with little experience and the results are often very inaccurate. Because the materials and measurements are new there is no oversight or perspective on these results and none of the international validation. The rush to follow fashion in this way is driven by the increasing need for (particularly) young faculty to perform at a high level and make a name for themselves in a field to progress their career. This is a dangerous trend in many ways but not the least is its encouragement of speed over rigour in the area of Thermophysics where care and time are essential.

We suggest that the fluid systems that are part of the project which is the subject of this workshop may well involve new materials and new chemistry/biochemistry. They therefore fall into the category identified above and therefore we advise very careful scrutiny of the data and its sources in process designs. We do not argue that the consequences of errors in properties will lead to designs that are physically dangerous but they may well not perform as expected thus tending to undermine your argument for their efficacy.

***Acknowledgements:*** *The authors are grateful to the organisers of the IProPBio First Workshop "ProGreS: Biomass selection, characterization and valorization" for the opportunity to contribute to their discussions and to prepare this paper. WAW wishes to thank Professor Roumiana Stateva of the Institute of Chemical Engineering, Bulgarian Academy of Sciences for fruitful collaboration over many years.*

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# *Biomass conversion processes: technologies and thermodynamic modeling*

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# Supercritical $CO_2$ extraction of feed stocks to generate high added value bio-products

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The huge amounts of waste generated every year by the industry, in addition to the great loss of valuable materials, pose serious organisational problems, both from an economic and ecological point of view. Many of the residues, however, have the potential to be reused and utilized completely via integrated bio-refining technologies. Extraction techniques which play a very important part in biorefineries should provide swift processing and yield quantitative recovery without degradation, complemented by easy separation of extracts from the solvent.

In this work, focussed on two generic biomass examples - grape seeds and spent coffee grounds, we outline the perspectives of introducing mild, efficient and with low environmental impact techniques that apply compressed fluids in biorefineries for obtaining high value-added products with a wide spectrum of applications.

**Key words:** $scCO_2$ extraction, biomass, valorisation bio-products, bioactive compounds

## INTRODUCTION

Diverse important natural sources of bioactive compounds (e.g. plants, fats, algae and other agricultural by-products and wastes) have been reported in the literature. The focus on plants as potential natural sources is ever present and new species are being continuously explored [1]–[7]. An increasing attention is paid to the use of waste biomass as a renewable resource of high added value compounds with applications in food, cosmetics, pharmaceutical industries, biodiesel production, etc. Recycling of waste and its valorisation to non-energy and energy related products is an effective and efficient way to resolve the serious problem with the huge amounts of waste generated daily, due to the increase, in both production and consumption [8], [9]. In view of this, the circular economy concept for biomass valorisation realized via integrated biorefineries [10]–[15] is becoming very important. A sustainable smart biorefinery would allow material processing and operation virtually waste free, based on alternative and/or mixed feed stocks, and would thus contribute environmental, social and financial benefits.

Figure 1 shows some of the options for production of food additives, antioxidants, flavonoids, enzymes and proteins, fatty acid esters, glycerol, etc. from diverse sources of raw material [8], [11].

Extraction techniques are a vital part of bio-refineries. Conventional extraction techniques, however, are time-consuming, require large amounts of toxic volatile organic compounds (VOCs) - solvents, and high energy spending. These shortcomings have generated interest in new advanced technologies that alleviate and/or eliminate the emission of pollutants to the environment. These green techniques are environmentally-compatible, sustainable, preserve natural resources and lower carbon footprints

## SUPERCRITICAL $CO_2$ EXTRACTION

In our days the use of organic sources to attain bioactive compounds requires the application of suitable, environmentally and ecologically friendly, innovative extraction techniques and processes. From this perspective, the methods of extraction should be technologically advanced and in line with the Green Chemistry principles related to all processes involved [16]–[18]. Supercritical fluids are gradually being used and endorsed to produce high value, natural bioactive substances from biological or organic wastes based raw materials. Supercritical fluids have the necessary transport properties that increase their ability to adjust. Moreover, supercritical fluids have low viscosity and disperse more easily within the solid matrix and have low surface tension, which allows rapid penetration of the solvent into the matrix thus increasing extraction efficiency [1], [2], [19].

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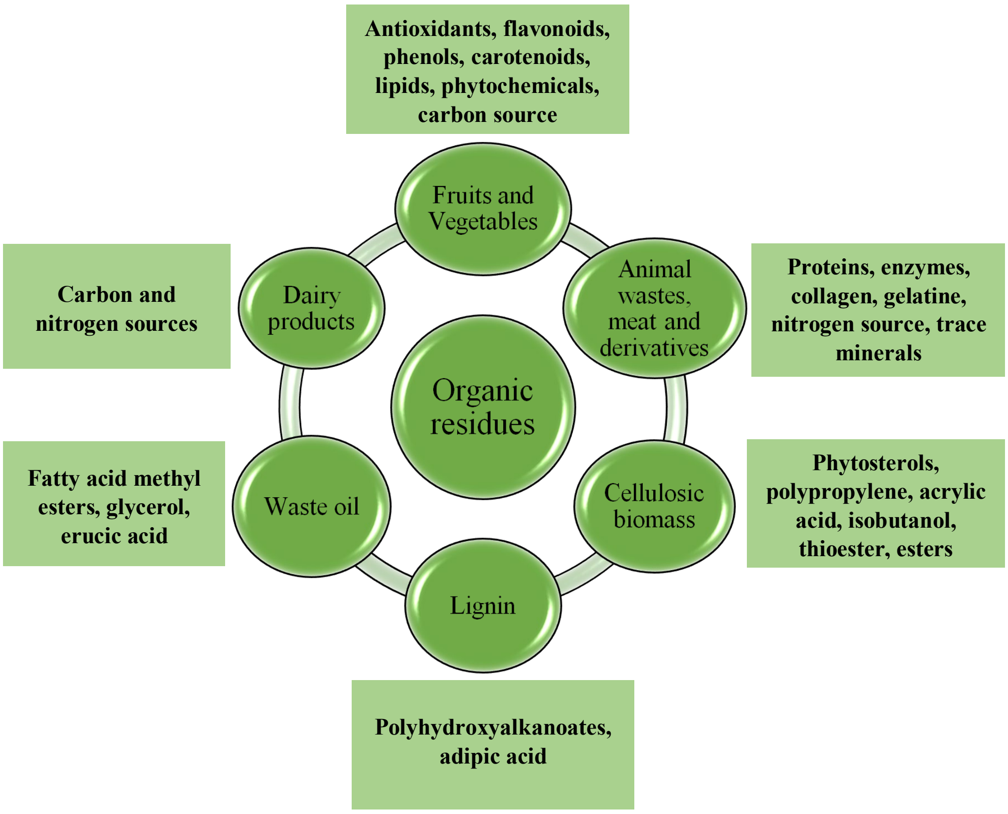

**Fig. 1.** Valorization of waste organic residues from several source of raw material.

Furthermore, because they are non-mutagenic and non-toxic are often referred to as the green solvents of the future. Supercritical $CO_2$ ($scCO_2$) is an excellent solvent, which is cheap and abundant and is principally used to isolate nonpolar bioactive compounds (carotenoids and lipids). One option to overtake this limitation, and to enhance the extraction of more polar compounds, such as flavonoids, is the addition of modifiers such as ethanol, water, ethyl lactate, or less green modifiers like methanol, and acetone [6], [19], [20].

Moreover, a combination of $scCO_2$ with sub-critical extraction methods, mainly pressurized liquid extraction (PLE), can be used as a very effective and considerable improvement of the conventional extraction to obtain a wide range of bioactive components from different natural and organic sources. In addition, there is the possibility to join the above process to other processes within the frame of a biorefinery [7].

It is understandable that the optimal extraction conditions will be different not only with respect to the solvent and matrix, but also - to the target components, which are often with very diverse chemical structures. Hence, great care should be taken to identify and adjust the operating conditions properly.

## GRAPE SEEDS AND SPENT COFFEE AS WASTE ORGANIC RESIDUES

Grape seeds (GS) and spent coffee grounds (SCG) wastes are two important feed stocks for development of biorefinery sustainability by circular production of high value-added products such as bioactive compounds, adsorbents and catalytic supports, polymers or nanocomposites, etc. along with products, which are of lower value but are utilized in huge quantities, such as biodiesel and other biofuels [14], [21]–[29]. Their properties complement each other, which opens up opportunities for optimization of the processes and product portfolios of sustainable biorefineries.

Grape is one of the largest fruit crops produced with an annual world production higher that 70 million tons [30] of which about 80 % is used for winemaking. Seed biomass (about 5 % of the whole grape), represents approximately (40 – 50) % of solid wastes and contains typically (8–15) % of oil.

Global coffee production creates huge volumes of waste per year. Around 650 kg of SCGs is produced from 1 ton of green coffee beans. Furthermore, during the preparation of 1 kg of soluble coffee approximately 2 kg of wet SCGs are obtained [31]. In 2014 around nine million tons of SCGs were dumped in landfills.

Extraction of the pressed GS or SCG with *n*-hexane is the current method applied to reuse the seeds biomass and to obtain the vegetable oil. Hexane is the usual solvent used in the laboratory and in industry. However, according to the European Chemicals Agency (ECHA) it is classified as a danger that may be fatal if swallowed or inhaled, as toxic to aquatic life with long lasting effects, and as a highly combustible liquid. It is suspected also of damaging fertility. Therefore, the green alternative extraction technique, applying supercritical $CO_2$ as a solvent, is a powerful option that can lower health and safety risks, and reduce environmental footprints.

## MATERIALS AND METHODS

Grape seeds and spent ground coffee were supplied by a wine industry and a coffee shop, with a particle size of 0.62±0.04 and 0.273±0.023 mm, respectively. The waste organic residues were dried for a period of 48 h at 343 K, to guarantee lowering excessive humidity which is harmful to $scCO_2$.

Supercritical extraction with $CO_2$, at flow rates of 0.11 kg/h, pressures up to 40 MPa, and temperatures up to 333 K, was performed in an apparatus, equipped with a 50 mL internal volume vessel, manufactured from AISI 316 stainless steel tubing (32 cm long with an internal diameter of 1.41 cm), following the previously described methodologies [17], [18], [24]. The conventional extraction used for comparison in our study, was carried out with *n*-hexane in a Soxhlet system.

The quantitative analysis of fatty acids distribution of the extracted esters was performed by transesterification of the glycerides in a 2M methanol solution of KOH, as recommended in the Annex I to Commission Regulation (EEC) No 2568/91(1). A GC-FID system with a fused-silica capillary column SP-2380, 60 m length, 0.25 mm of internal diameter and 0.20 μm film thickness, with helium as the carrier gas at a constant flow rate of 1.0 mL/min was employed for the analysis of the fatty acid methyl esters (FAMEs) [24].

## RESULTS AND DISCUSSION

The effect of the $scCO_2$ operating parameters - pressure and temperature, on the extraction yields, as well as a comparison with the *n*-hexane extraction yield, can be deduced from Table 1.

The results of applying the $scCO_2$ technique, confirmed that at the same temperature the extraction time, needed to achieve the maximum yield is influenced by pressure. At higher temperatures this trend is more pronounced with the extraction time decreasing considerably with increasing pressure. This behavior is explained by the rise in solvent density, which leads to enhancing the solvation power of $CO_2$ [31]. It should be noted that, considering the standard deviation, the maximum oil yields achieved by the $scCO_2$ extraction were, for both matrices, within the range of the *n*-hexane extraction oil yield. However, the $scCO_2$ times to obtain the maximum yields, compared to *n*-hexane extraction, were shorter - around 110 – 130 min for the higher pressure (40 MPa) and 200 min – for the case when pressure was 30 MPa (Table 1).

Figures 2 and 3 show the main fatty acid composition (% of total fatty acids) results of the GS and SCG oils, obtained by *n*-hexane and $scCO_2$ extraction. Fatty acids like myristic (C14:0), palmitoleic (C16:1), gadoleic (C20:1), behenic (C22:0), nervonic (C24:0) are also present in the oil but in very low quantities – to the total of less than 1.3 %.

According to Figures 2 and 3, the distribution of the fatty acids in the extracted oils does not depend significantly on the extraction method. The main fatty acid in both oils is linoleic (C18:2). However, the grape seed oil contains more linoleic acid esters (64 - 67 %) when compared to the SCG oil (41 - 43 %). On the other hand, the amounts of palmitic acid (C16:0) in the first and the second oil types are 8 and ≈ 33%, respectively. These results are confirmed also by the fact that monounsaturated MUFA and di-unsaturated DUFA are the prevailing compounds in both oils, when compared to the saturated. However, the two oil types are complementing each other, for instance as biorefinery feed stocks, since the grape seeds oil is significantly more unsaturated (with ≈86 % in total) in comparison with the SCG oil (around 56 %).

## CONCLUSION

The effects of the $scCO_2$ technique operating parameters – temperature and pressure, on the yield and the fatty acid profile of the oils extracted from grape seeds and from spent ground coffee biomass were evaluated in detail and reported.

Taking into consideration the higher amounts of essential MUFA and DUFA, the grape seeds oils can be designated to human consumption. In biodiesel application, after separating high value bioactive compounds like diterpenes, caffeine, polyols, etc., mixtures of the two types of oils can provide properties like cetane number, lubricity, cold filter plugging points, etc. with complementing qualities.

**Table 1.** Oil yields and extraction times, applying scCO2, as a function of the operating conditions. Comparison to *n*-hexane extraction results.

| scCO$_2$ conditions | Extraction method | Grape seeds Oil Yield (%) | Time (min) | Spent coffee Oil Yield (%) | Time (min) |
|---|---|---|---|---|---|
| $p$(MPa)/$T$ (K);<br>CO$_2$, flow rate 0.11 kg/h | *n*-hexane | 12.28±0.35 | 240 | 10.38±0.82 | 240 |
| | 30/313 | 11.96±0.60 | 191 | 10.60±0.54 | 191 |
| | 40/313 | 12.07±0.55 | 148 | 10.10±0.72 | 110 |
| | 30/333 | 12.17±0.38 | 214 | 9.41±0.59 | 223 |
| | 40/333 | 12.83±0.56 | 134 | 10.11±0.65 | 110 |

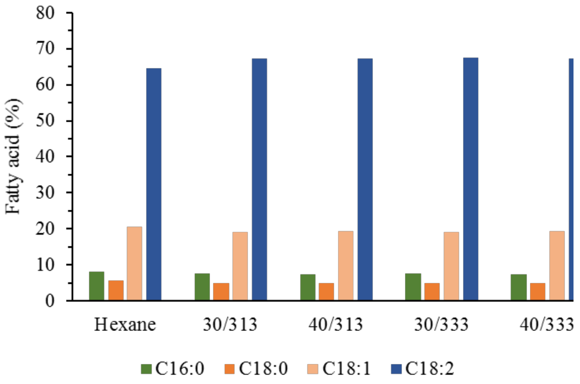

**Fig. 2.** Fatty acid composition (% of total fatty acids) GC-FID analysis of GS oils obtained by hexane and scCO$_2$ extraction $p$(MPa)/T (K).

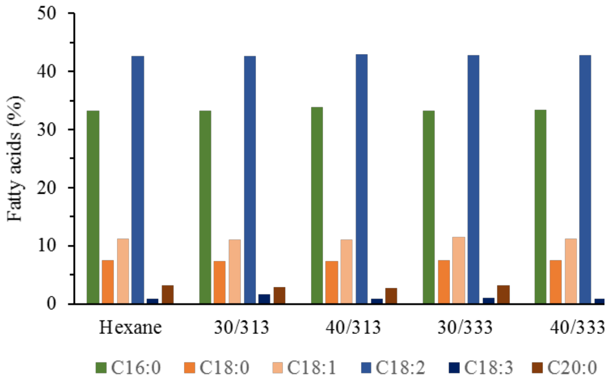

**Fig. 3.** Fatty acid composition (% of total fatty acids) GC-FID analysis of SCG oils obtained by hexane and scCO$_2$ extraction $p$(MPa)/T (K).

The scCO$_2$ oil extracts, as compared to those from the conventional *n*-hexane extraction, are obtained by an integrated technique with reduced time for processing. They do not require use/regeneration of harmful solvents and generation of hazardous wastes. Furthermore, extraction with scCO$_2$ has low environmental impact, and in combination with similar techniques are a prerequisite for circular waste-free production in sustainable smart biorefineries.

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# Modelling the kinetics of supercritical $CO_2$ extraction of biomass

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This work addresses the modelling and simulation of the kinetics of $CO_2$ supercritical extraction of oils from biomass. Experimental and simulation results from different matrices and models are presented.

gPROMS Model Builder is used to find solutions to three different models applied to study the extraction of three different volatile oils from aromatic plants (coriander, fennel and savoury), and from a bioresidue, industrial grape seeds. The supercritical extraction experiments performed at different temperature, pressure and flow rate conditions provide the data to the modelling studies and for model parameter estimation. The qualitative and quantitative agreement between the experimental and simulated extraction profiles in terms of yields was good for the cases investigated.

**Key words:** supercritical $CO_2$ extraction, extraction kinetics, mathematical modelling, simulation, biomass valorisation.

## INTRODUCTION

Nowadays, increasing attention is being drawn to the effective use of waste biomass and vegetal material as renewable sources of valuable compounds with applications in several industries, as food, cosmetics, pharmaceutical, biodiesel production, etc. While the extraction of oils from aromatic plants is well established [1–4], the use of by-products or biowastes is still underexplored. Seed biomass from *Vitis vinifera* L. is an example of underutilized biowaste. With an oil content of (8 - 15) % (w/w), rich in long chain polyunsaturated fatty acids (PUFAs) and antioxidants [5,6], and representing about (20 - 25) % of the biomass generated by the wine industry, it is still considered a disposable material and rarely valorised.

Extraction with supercritical $CO_2$ (SCE) has the great advantage of preventing or, at least, minimizing the degradation of bioactive compounds present in the matrix to be extracted due to the comparatively low temperatures used and oxygen free atmospheres. Furthermore, it allows obtaining solvent-free products and, unsurprisingly, is currently establishing itself as the viable, sustainable and eco-compatible alternative to the use of organic solvents. Yet, kinetic data are not abundant, and, for some systems they are scarce and superficial.

Dynamic models are a useful tool for the design, optimization and scale-up of supercritical fluid extraction processes from laboratory to pilot and industrial scales.

In particular, mass balance based models which include mass transfer coefficients in fluid and/or solid phases have a strong physical significance. They take into account the characteristics of the plant matrix, namely the particle size, the bed porosity and also the equilibrium relationships and mass transfer mechanisms.

Although several models have been proposed in the literature, their solution is not always trivial and, additionally, the estimation of some parameters using experimental data is required. Within this context, the opportunity to use new tools to model, simulate and perform parameter estimation seems promising.

In view of the above, the aim of our work is to model the kinetics of the SCE of oils from aromatic plants, namely, coriander, fennel, and savoury, and from a biowaste - industrial grape seeds, obtained directly from a Portuguese industry, by applying an efficient solution method to different models.

## METHODOLOGY

The extraction conditions and the experimental results were previously reported for the aromatic plants [7] and the grape seeds [8]. For the aromatic plants, the simulation results presented by Grosso et al. [7] are used as comparison in order to evaluate the efficiency of the solution method used in this work.

The models were implemented in gPROMS ModelBuilder [9], an equation-oriented modelling

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and optimisation platform for steady-state and dynamic systems, and the experimental data was used to obtain the model parameters using gPROMS parameter estimation.

The desorption model by Tan and Liou [10] and the model proposed by Sovová [11], both without axial dispersion are used for the aromatic plants. These models consider the variation of the concentration of the supercritical fluid as it flows along the extractor and, thus, include partial differential equations.

The model by Tan and Liou [10], from now on referred to as model 1, is described by:

$$\frac{\partial C}{\partial t}+\frac{u}{\varepsilon}\frac{\partial C}{\partial h}+\frac{1-\varepsilon}{\varepsilon}\frac{\rho_s}{\rho_f}\frac{\partial q}{\partial t}=0 \tag{1}$$

$$\frac{\partial q}{\partial t}=-k_d q \tag{2}$$

With the initial and boundary conditions defined by:

$$q(h,0)=q_0 \tag{3}$$

$$C(h,0)=C_0 \tag{4}$$

$$C(0,t)=0 \tag{5}$$

Where $C$ is the concentration in the fluid phase, $t$ is time in minutes, $u$ is the superficial velocity of supercritical fluid ($m \cdot s^{-1}$), $\varepsilon$ is the bed void fraction, $\rho_s$ and $\rho_f$ are the density of the solid and fluid phase ($kg \cdot m^{-3}$), $q$ is the solute concentration in the solid phase ($kg \cdot kg^{-1}$) and $k_d$ is the desorption coefficient ($s^{-1}$).

The model by Sovová [11], from now on referred to as model 2, is described by:

$$\frac{\partial C}{\partial t}+\frac{u}{\varepsilon}\frac{\partial C}{\partial h}+\frac{1-\varepsilon}{\varepsilon}\frac{\rho_s}{\rho_f}\frac{\partial q}{\partial t}=0 \tag{6}$$

$$\frac{\partial q}{\partial t}=-\frac{k_f a \rho_f}{\rho_s}(C_0-C)\,, \qquad q\geq q_k \tag{7}$$

$$\frac{\partial q}{\partial t}=-k_s a q\ , \qquad q<q_k \tag{8}$$

With the following initial and boundary conditions:

$$q(h,0)=q_0 \tag{9}$$

$$C(h,0)=C_0 \tag{10}$$

$$C(0,t)=0 \tag{11}$$

Where $q_k$ is the initial content of the difficult accessible solute in the solid ($kg \cdot kg^{-1}$), $a$ is the surface of a unit volume of particles ($m^{-1}$), and $k_s$ and $k_f$ are the internal and external mass transfer coefficients.

The third model used in this work, model 3, developed by Sovová and Stateva [12], is described by the following set of equations:

$$\frac{dw}{dt}+\frac{w}{t_r}=\frac{k_f a_0}{\varepsilon}\left(w^+-w\right) \tag{12}$$

$$\frac{dw_s}{dt}=-q' t_r\frac{k_f a_0}{\varepsilon}\left(w^+-w\right) \tag{13}$$

$$w^+=Kw_s+\frac{w_s^b}{w_t^b+w_s^b}(w_{sat}-Kw_s) \tag{14}$$

With the initial conditions:

$$w(0)=w_0 \tag{15}$$

$$w_s(0)=w_{s,0} \tag{16}$$

The yield, $e$ ($kg \cdot kg^{-1}$ solid), is defined by:

$$e=q'\int_0^t e\,dt \tag{17}$$

$$e(0)=0 \tag{18}$$

Where $w$ is the oil concentration in the fluid phase inside the extractor ($kg \cdot kg^{-1}$ $CO_2$), $w_s$ is the oil concentration in the solid phase ($kg \cdot kg^{-1}$ solid), $t$ and $t_r$ are the extraction and residence time (min), respectively, $q'$ is the specific flow rate (kg $CO_2 \cdot min^{-1} \cdot kg^{-1}$ solid), $w^+$ is the oil concentration at solid-fluid interface ($kg \cdot kg^{-1}$ $CO_2$), $\varepsilon$ is the void fraction in the bed, $k_f\,a_0$ ($min^{-1}$) is the volumetric fluid phase mass transfer resistance, $K$ is the partition coefficient, $w_t$ ($kg \cdot kg^{-1}$ $CO_2$) is the monolayer adsorption maximum content, $w_{sat}$ ($kg \cdot kg^{-1}$ $CO_2$) is the solubility of the free oil compound, and $b$ is a coefficient that should be higher than one.

This model considers homogeneous concentration in the extractor at both solid and fluid phases and assumes that the extracts are located on the surface of the solid particles. Thus, the extracts overcome only the external mass transfer resistance, which is usually much smaller than the internal resistance. This assumption allows neglecting internal diffusion, which is compatible with finely ground substrates where the diffusion path in the particles is short and the extract is easily accessible, resulting in negligible internal mass transfer resistance.

The grape seeds are very complex mixtures of mainly triacylglycerols (TAG) with minor amounts of other compounds. Due to this complexity the grape seeds oil is usually represented by a single model TAG, and, for the purposes of modelling and comparison with the results of other authors [8] triolein was selected to exemplify the oil. Model 3 requires solubility data of the model TAG in the supercritical $CO_2$, which was calculated applying the predictive Soave-Redlich-Kwong (PSRK) cubic EoS [13].

gPROMS Modelbuilder parameter estimation with MAXLKHD solver was used to estimate the desorption rate constant ($k_d$) for model 1, the internal and external mass transfer coefficients ($k_s$ and $k_f$) for model 2, and the partition coefficient ($K$) for model 3. gPROMS uses a maximum likelihood parameter estimation problem and attempts to determine values for the uncertain physical and variance model parameters that maximize the probability that the mathematical model will adequately predict the values obtained from the experiments.

To apply model 3 to grape seeds, the value of $k_f$ was calculated following [8], where the relation proposed by [14] was used. The values obtained were 6.77E-4 and 6.95E-4 for $T$ = (313 and 333) K, respectively. Parameter $b$ was set to 7 [12] and $w_t$ was set to 60% of the initial concentration of the oil in the grape seeds.

In order to compare the fitting accuracy obtained with other works, two standard deviation measures were calculated after parameter estimation: the absolute average relative deviation, AARD, defined by Eq. (19), and the root mean square deviation, RMSE, defined by Eq. (20), where $N$ is the total number of experimental points, and $e_i^{exp}$ and $e_i^{est}$ - the $i$-th experimental and estimated point, respectively.

$$\mathrm{AARD} = \frac{100}{\mathrm{N}}\sum_{j=1}^{N}\frac{\left|e_i^{exp} - e_i^{est}\right|}{e_i^{exp}} \qquad (19)$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\left(e_i^{exp} - e_i^{est}\right)^2} \qquad (20)$$

## RESULTS AND DISCUSSION

After the estimation of the parameters for each of the models used ($k_d$ for model 1, $k_s$ and $k_f$ for model 2 and $K$ for model 3), an analysis of the yield profiles was performed. The quality of the fitting obtained with parameter estimation was also evaluated and, for the aromatic plants, compared with previously reported results [7]. The yield profiles are presented as a set of data points, obtained experimentally and with parameter estimation. The lines connecting the points were included to improve readability and do not represent simulation results.

On Figure 1 the yield of extraction *versus* time is shown for coriander. The experimental data, the simulation results reported in [7] and the ones obtained in this work using the models from Sovová, and Tan and Liou are depicted. Although some limitations were encountered in parameter estimations due to the limited amount of data available, the results obtained improved the ones previously reported for the aromatic plants [7], as assessed by the absolute deviation error (Table 1).

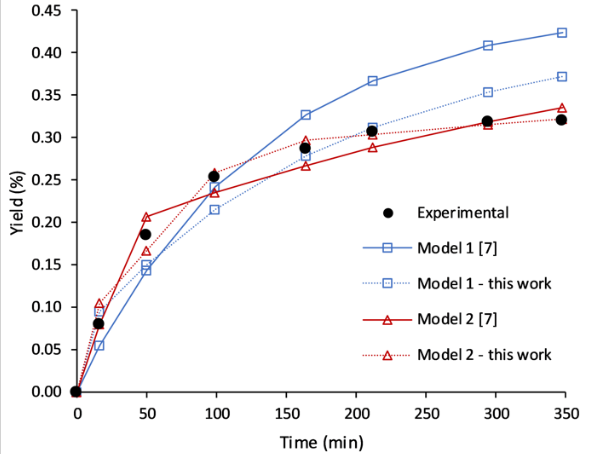

**Fig. 1.** Experimental and simulated extraction profiles for coriander P = 9 MPa, T = 313 K, $P_{size}$ = 0.6 mm, F = 6 L/min.

The results for savoury using model 1 and 2 are displayed in Figure 3. Model 2 performs better, when compared to model 1, with a lower AARD (Table 1), and the results obtained in this work reduce the AARD by approximately 50 %.

For the fennel system (Figure 4), the AARD previously reported [7] is quite small for model 2, and could not be improved further. A reason for not improving might be that the AARD has reached the experimental reproducibility of the data being modelled.

**Table 1.** Parameter estimation results for the systems studied

| System | Model | $P$ (MPa) | $T$ (K) | $F$ (L·min$^{-1}$) | $P_{size}$ (mm) | $k_d$ (s$^{-1}$) | $k_f$ (m·s$^{-1}$) | $k_s$ (m·s$^{-1}$) | $K$ | RMSE (%) | AARD (%) | AARD (%) [7] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coriander | 1 | 9 | 313 | 6 | 0.6 | 5.1E-05 | | | | 2.9E-02 | 10.7 | 19.0 |
| Coriander | 1 | 9 | 313 | 8 | 0.6 | 1.7E-04 | | | | 2.6E-02 | 7.1 | 14.9 |
| Coriander | 2 | 9 | 313 | 6 | 0.6 | | 1.8E-07 | 2.6E-09 | | 1.2E-02 | 6.3 | 5.3 |
| Coriander | 2 | 9 | 313 | 8 | 0.6 | | 2.3E-06 | 1.3E-08 | | 1.5E-02 | 3.3 | 3.2 |
| Savoury | 1 | 9 | 313 | 6 | 0.6 | 1.7E-04 | | | | 2.9E-01 | 9.3 | 18.7 |
| Savoury | 2 | 9 | 313 | 6 | 0.6 | | 8.2E-03 | 4.6E-08 | | 6.0E-02 | 3.4 | 6.5 |
| Fennel | 1 | 9 | 313 | 6 | 0.6 | 2.4E-04 | | | | 1.5E+00 | 7.3 | 10.0 |
| Fennel | 2 | 9 | 313 | 6 | 0.6 | | 9.8E-06 | 9.3E-08 | | 1.0E-01 | 3.6 | 2.1 |
| Grape seeds | 1 | 40 | 313 | 1 | 0.6 | 1.4E-04 | | | | 2.4E+00 | 26.7 | |
| Grape seeds | 1 | 40 | 333 | 1 | 0.6 | 1.6E-04 | | | | 2.6E+00 | 28.7 | |
| Grape seeds | 3 | 40 | 313 | 1 | 0.6 | | | | 0.22 | 5.0E-01 | 4.5 | |
| Grape seeds | 3 | 40 | 333 | 1 | 0.6 | | | | 0.43 | 7.0E-01 | 8.6 | |

Figure 2 depicts the extraction profiles with two different flows of supercritical $CO_2$, and an increase in the speed of extraction is observed when the flow increases. Once again, an improvement in the fitting was achieved with the methodology used in this work.

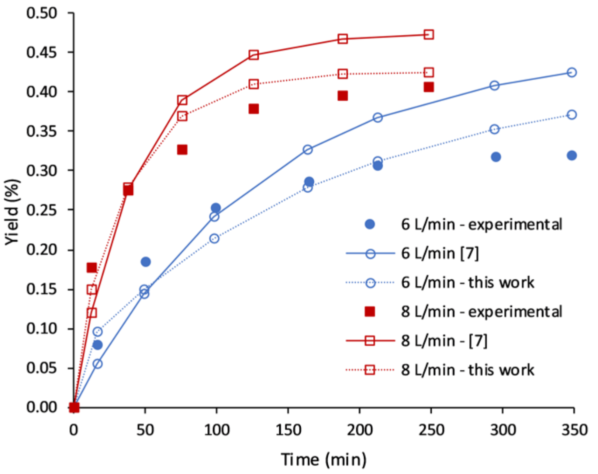

**Fig. 2.** Extraction profiles for coriander using model 1. P = 9 MPa, T = 313 K, $P_{size}$ = 0.6 mm, F = (6 and 8) L/min.

The results obtained demonstrate the good performance of gPROMS ModelBuilder, which was able to improve the AARD for almost all cases investigated.

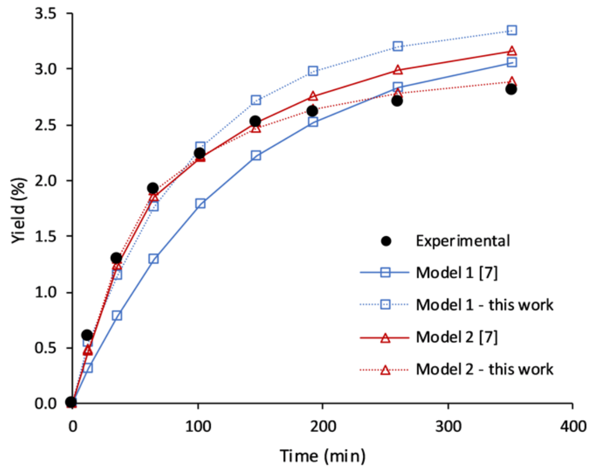

**Fig. 3.** Experimental and simulated extraction profiles for savoury. P = 9 MPa, T = 313 K, $P_{size}$ = 0.6 mm F = 6 L/min.

Figure 5 shows the profiles for the grape seeds extraction, where the temperature is different for each case. The increase in temperature results in a faster extraction.

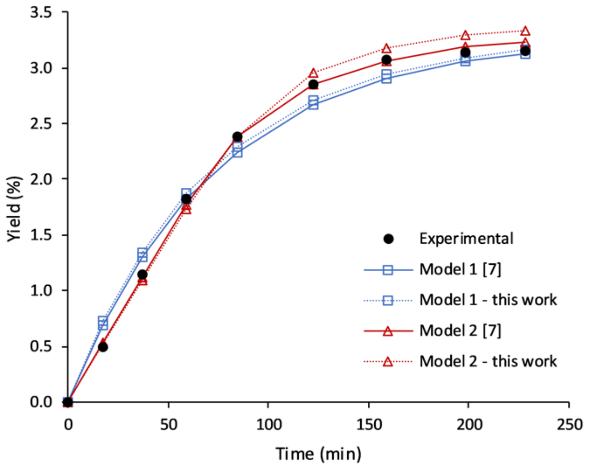

**Fig. 4.** Experimental and simulated extraction profiles for fennel $P$ = 9 MPa, $T$ = 313 K, $P_{size}$ = 0.6 mm $F$ = 6 L/min.

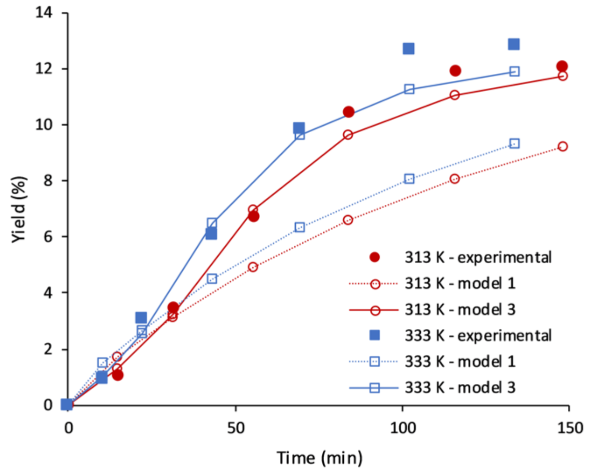

**Fig. 5.** Experimental and simulated extraction profiles for grape seeds using models 1 and 3. P = 40 MPa, $P_{size}$ = 0.6 mm F = 1 L/min, T = (313 and 333) K.

Model 3 is much more effective fitting the experimental data than model 1, for both cases. Indeed, the high AARD obtained with model 1, indicates that this model is not adequate for grape seeds extraction simulation. Model 3 incorporates in a rigorous way the interplay between phase equilibria (solubility) and kinetics, and the results obtained demonstrate that albeit the simplifications introduced in representing the grape seeds oils by just a single model TAG, there is a good qualitative and quantitative agreement between the experimental and calculated extraction yields at the SCEs operating conditions examined. It should be emphasized that although the internal diffusion is neglected, the model can still deliver adequate results, providing that the particle size is small, as is the case for the matrix used in this work.

## CONCLUSIONS

This work presents the results from modelling the kinetics of SCE of biomass from three aromatic plants and industrial grape seeds, obtained directly from a Portuguese industry without preliminary treatment. The influence of the operating conditions on the extraction yield was analysed for some of the systems and reported.

To simulate the extraction kinetics, three different models were used. The model equations were integrated using gPROMS ModelBuilder and the results were compared to previously reported data. Models 1 and 2 are show to be adequate for the aromatic plants investigated, with AARD values in the range (3.3 – 10.7) %. However, they could not simulate adequately the grape seeds kinetics, as demonstrated by the large AARD obtained. For model 3, the qualitative and quantitative agreement between the experimental and simulated extraction profiles in terms of yields for the grape seeds was quite adequate taking into consideration the complex nature of the systems examined. The good fitting results also indicate that the model can be successfully used in finely ground matrices where the internal diffusion contribution to the extraction phenomena is very small and, thus, negligible.

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# Extraction and characterization of Burdock extracts (leaves, seeds and roots) with compressed solvents technologies

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In this study, an advanced green extraction technique applying supercritical $CO_2$ ($scCO_2$) and $scCO_2$ with co-solvent (esters, alcohols or hydroalcoholic solutions) is used to obtain value added compounds from the aerial (leaves and seeds) and underground (roots) parts of *Arctium lappa*, commonly known as burdock. In order to increase the yields multi-step $scCO_2$ extraction was also tested. Thus, the highest yield of 12.78 wt % for *A. lappa* leaves was achieved by a six-step sequential $scCO_2$ extraction with ethanol as a co-solvent, for *A. lappa* roots - 32.82 wt % by a three-step sequential $scCO_2$ extraction with hydroalcoholic solution (methanol-water), while for the *A. lappa* seeds - 19.02 wt % by using $scCO_2$ with ethanol as co-solvent. Finally, the effectiveness of the above techniques with that of a conventional Soxhlet extraction with regard to yield was compared.

**Keywords:** *Arctium lappa*; supercritical $CO_2$ extraction; co-solvent; multi-step extraction.

## INTRODUCTION

Presently, more and more people turn away from modern medicine and look for solutions to their health problems in traditional medicine and natural products [1]. One such example is the *Arctium lappa* plant, more commonly known as burdock. It is native to the Eurasian region but due to its rapid growth it has spread to other parts of the world such as South America where it is considered an invasive species [2]. Though not a very common dish in most countries it is cultivated and regularly used in East Asian cuisine [3-5], while in the UK, Dandelion and burdock is a popular and widely consumed beverage.

The roots are the edible part of the plant, however, it has been demonstrated that the seeds and leaves contain compounds with antioxidant [6-10], antibacterial [11,12] and anti-inflammatory biological activities [13,14]. Hence, recently some considerable effort has been invested into their obtainment, characterization and applications [6,7,9-17].

The aim of the present study is to examine the capabilities of mild and green techniques that apply a supercritical solvent without and with co-solvents to obtain high value compounds from the different parts of the burdock plant, which nowadays is treated as a waste, with the view of its complete valorization and in accordance to the principles of the circular economy.

**Fig. 1.** *Arctium lappa* [18]

## MATERIALS AND METHODS

### *Sample preparation*

The burdock seeds were supplied by a producer from Ivaiporã city, state of Paraná, Brazil (GPS location: 24°14’47.4”S 51°40’32.8” W), in 2018. The material was dried, milled and separated using

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Tyler series sieves of different mesh size in a mechanical shaker. The plant material used for all experiments had particle size of (0.41 – 0.71) mm.

### *Soxhlet extraction*

Soxhlet extraction was performed on all three parts of the burdock plant – the seeds, leaves and roots. All extractions were done in triplets and the yields given below are the average yields. These results served as references in our further investigations. The yield of each extraction was calculated according to:

$$Yield(\%) = \frac{mass\ of\ extract\ (g)}{mass\ of\ sample} * 100 \qquad (1)$$

### *Supercritical fluid extraction*

All supercritical fluid extractions (SFE) were performed in two identical, home-made laboratory extraction units at LACTA laboratory at UFPR – Brazil (Fig. 2.).

The specifics of the SFE units are extensively described in previous works of the research group [19-25]. In summary, the extraction equipment used consists of an extraction vessel equipped with temperature-regulation jacked with internal volume of $62.4\cdot10^{-6}$ m$^3$ ($\varnothing = 1.9\cdot10^{-3}$ m and $L = 22.0\cdot10^{-3}$ m). The pressure is controlled by a syringe pump and monitored by a manometer, while the actual flow rate of the dynamic extraction was controlled by a needle valve (V5, Fig. 2.).

The extraction procedure involved: Firstly, the sample was loaded within the vessel. In the experiments with a co-solvent, the latter was mixed with the sample before loading it into the extraction vessel. Then, after each step of the sequential extractions, the top of the extraction vessel was opened and fresh co-solvent was introduced.

When the desired pressure of the syringe pump was reached, the gas was introduced into the extraction vessel. When pressure equilibrium was attained, the static extraction started (60 minutes for all experiments), after which the dynamic extraction was performed by opening Valves 4 and 5 at a flow rate of 2 ml·min$^{-1}$. The extract was collected in glass vials and weighed to determine the extraction yield. In the cases when a co-solvent was used it was evaporated before weighing the yield.

## RESULTS AND DISCUSSIONS

On all samples firstly a conventional Soxhlet extraction was applied. The results obtained served as a reference for the consecutive yields comparison. All experiments were performed in triplets and at a fixed extraction time (360 minutes) with the following solvents:

- leaves – water;
- seeds – methanol, ethanol, ethyl acetate and hexane;
- roots – methanol, ethanol, ethyl acetate, hexane and water.

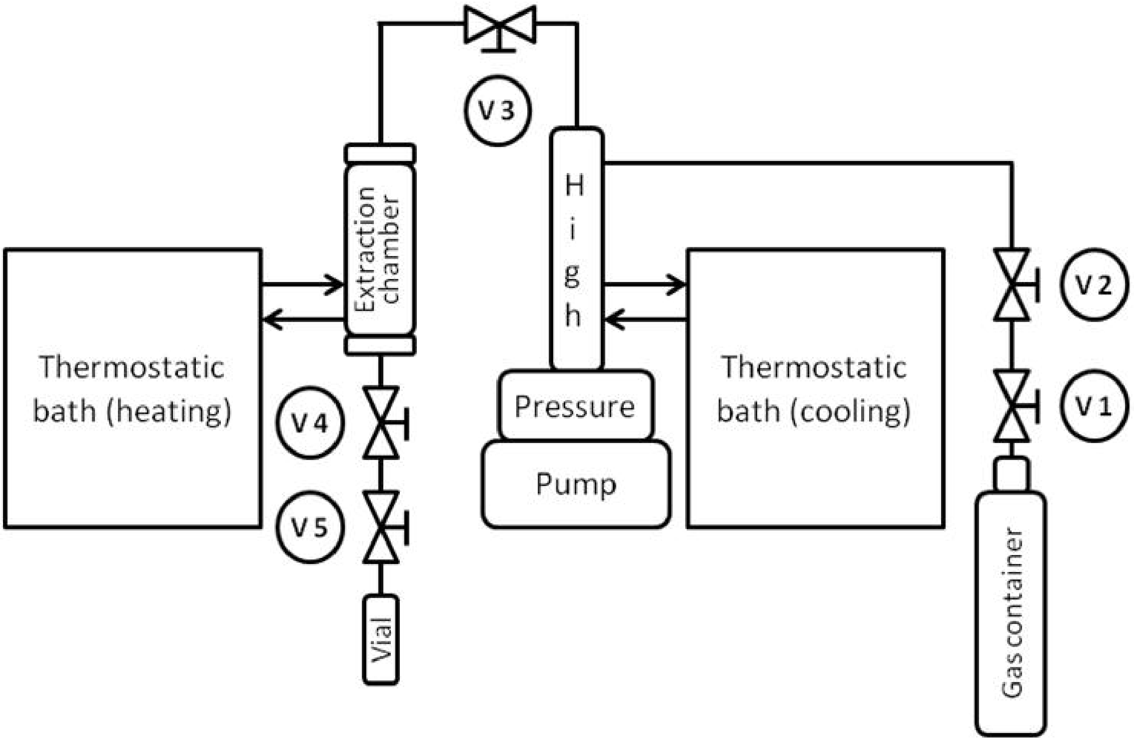

**Fig. 2.** Schematic representation of the supercritical fluid extraction equipment used

The extraction yield for the leaves was 33.58 wt %, while the highest yields for the seeds and roots were 40.88 wt % with ethanol and 35.44 wt % with water, respectively. The second highest yield for the roots was achieved applying methanol (21.76 wt % – over 3 times higher than that with the third solvent, ethanol). This result was used as a basis for further scCO$_2$ extraction with a hydroalcoholic co-solvent (methanol-water).

Extractions with scCO$_2$ followed and it was demonstrated that for the seeds the average yield was 6.45 wt %. At the conditions applied, and because of the low oil content in the leaves and roots, supercritical CO$_2$ extraction seems to be unsuitable for those materials, so scCO$_2$ extractions with ester, alcoholic and hydroalcoholic co-solvents were carried out, increasing thus the yields significantly.

The choice of the co-solvents was based on the yields achieved applying the Soxhlet extractions and were different for the different plant matrices. Additionally for the leaves and the roots, sequential scCO$_2$ extractions with a co-solvent were carried out to determine the effects of a single-step and multi-step extractions on the yield.

The co-solvents for the seeds were ethanol and ethyl acetate at a co-solvent to sample mass ratio of 2:1 and yielded 19.02 wt % and 13.40 wt %, respectively.

Methanol was used as a co-solvent for the experiments with roots and the yield was 4.13 wt % for the single-step extraction, and 5.65 wt % for the three-step one, respectively.

For the leaves ethanol was used as a co-solvent and the yield for the single-step extraction was 6.10 wt %, while that for the six-step sequential extraction was 12.78 wt % (Fig. 3.).

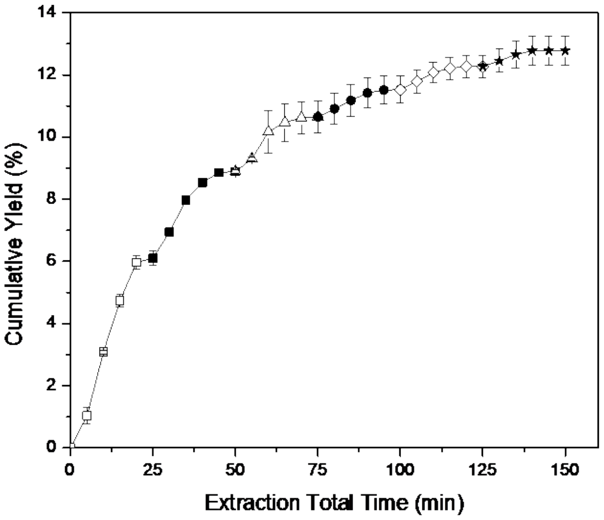

**Fig. 3.** Kinetic curves of a six-step sequential scCO$_2$ extraction with ethanol as a co-solvent: step 1 - □; step 2 - ■; step 3 - Δ; step 4 - ●; step 5 - ◇; step 6 - ★.

Additional extractions with a hydroalcoholic solution were carried out for the roots and the leaves. The yields obtained were 2.40 wt % for the roots with methanol-water solution and 9.12 wt % for the leaves with ethanol-water solution.

Due to the unexpected low yield for the roots, a three-step sequential extraction was carried out with the same solvent. The results obtained showed a considerable increase in the yield - 1.37 wt % for the first step, 20.22 wt % for the second step and 11.23 wt % for the third one, with a 32.82 wt % cumulative yield.

We attribute this extremely low yield of the initial step to difficulties for the solvent to penetrate and break the cellular walls of the samples.

GC–MS analyses were performed for all burdock roots extracts [23]. The major compounds found were diisooctyl phthalate (DIOP) and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), glycerol, methyl oleate, butanoic acid and pentadecanal.

For the *A. lappa* leaves, the phytochemical compounds profile obtained from thin layer chromatography revealed the presence of lactones, terpenoids, and esters in extracts [7]. Furthermore, the DPPH and the phosphomolybdenum reduction methods found high values of antioxidant activity in the extracts, and a number of important phenolic compounds like lupeol acetate, amyrin acetate, diisooctyl phthalate and phytol were identified by GC analysis [7].

## CONCLUSIONS

The present work investigates the potential of green techniques for extraction of value added compounds from *A. lappa* leaves, seeds and roots as alternatives to conventional extractions using large quantities of organic solvent.

The highest yield obtained for the leaves – 12.78 wt % was achieved by a sequential scCO$_2$ extraction with ethanol as co-solvent. For the seeds the highest yield was obtained by scCO$_2$ with ethanol as a co-solvent – 19.02 wt %, while for the roots a yield of 32.82 wt % was achieved applying scCO$_2$ with a hydroalcoholic solution (methanol-water) in a three-step sequential extraction.

These results prove that extraction with scCO$_2$ plus co-solvents is a viable green alternative to conventional extraction techniques, which allows obtainment of high value substances with a wide spectrum of applications. Furthermore, the application of supercritical extractions processes within a biorefinery will pave the way to valorization of burdock – a plant that is considered an invasive weed in the majority of countries and is highly underused at present.

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# Extraction of lipids from waste biomass using compressed solvents: Kinetic and thermodynamic aspects

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Traditional oil extraction involves organic solvents and represents both health and environmental concerns; besides, the large spend of these solvents is costly. Thus, safer alternative methods of extractions arouse interest. Supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and gas-expanded liquid (GXL) extraction can be applied to a full range of matrices. This paper aimed to briefly discuss some aspects related to the oil extraction from different biomasses using $scCO_2$ + organic solvents extraction technique in semi-batch scheme and highlight some kinetic and thermodynamic aspects of this promising extraction strategy.

**Key words:** Biomass oil extraction, high-pressure, supercritical $CO_2$, co-solvent, GXL extraction.

## INTRODUCTION

Application of organic solvents for liquid extraction purposes brings risks to the environment and public health. Therefore, alternative extraction processes that might link high yield to economic viability are of great interest. Recently, pressurized liquid extraction (PLE), gas-expanded liquid (GXL) extraction, and supercritical fluid extraction (SFE) gained attention due to their green appeal. These extraction methods have as main advantages the elimination of large amounts of solvent, high selectivity, and the facilitated separation between solvent and solute. [1–3].

The SFE has been applied to several plant matrices, reducing the need for downstream process steps, by eliminating the solvent from the extracts, and providing flexibility in process conditions with the addition of different co-solvents to modulate selectivity and yield [4,5]. In this matter, supercritical carbon dioxide plays an important role due to its mild critical point conditions, around 31 °C and 74 bar, furthermore, it is an inexpensive, highly available, non-flammable, and non-toxic gas [6]. Another extraction technique that arises interest is the pressurized liquid extraction, in which higher temperatures are achieved, elevating the solubility of oils in the solvent and lowering the viscosity and the surface tension, leading to shorter extraction times and lower solvent spent compared to batch extraction process [7].

Gas-expanded liquid (GXL) extraction represents a transition between the SFE and the PLE. In this technique, a compressible gas is blended into the extraction liquid, reducing mass transfer effects by lowering the viscosity and increasing the density of the solvent. For safety and economic reasons, it is usual to perform carbon dioxide-expanded liquids (CXL) extraction modulating the pressure and $CO_2$ amount to achieve better solvation properties. In comparison with the use of $scCO_2$, the main advantage of CXL is related to the possibility of applying lower pressure operations and, when compared to PLE, the solvent usage is reduced [8,9].

Oil extraction from spent coffee grounds was investigated by Couto *et al.* [12] at various conditions, between 40 and 55 °C, and 15 and 30 MPa. After 3h of extraction, the highest yield of 15.4 % was obtained at 25.0 MPa and 50 °C, corresponding to 85 % of the total oil content of spent coffee grounds. The use of 6.5 wt% ethanol in $scCO_2$ extraction system reduced the extraction time to roughly one third and diminished the solvent usage to acquire a 12.9% extraction yield. Andrade *et al.* [13] studied the extraction of spent coffee grounds at 40, 50 and 60 ºC and 10 to 30 MPa during 2.5 h, and reported a maximum yield of 10.5 % with $scCO_2$ only, and 15 % in the presence of ethanol as co-solvent at otherwise identical conditions.

Extraction of rice bran lipids was performed by Sparks *et al.* [14], using pressurized liquid propane and supercritical carbon dioxide. The maximal yield of oil achieved by them using $scCO_2$ was 0.222 kg per kg of rice bran for conditions of 45°C and 35 MPa, and the maximal yield achieved with propane was 0.224 kg per kg of rice bran at 0.76 MPa and ambient temperature. These results correspond to approximately 85% of the total oil content on the raw material.

Soares *et al.* [15] compared the extraction of rice bran oil using $scCO_2$ (at 40 to 80 °C and 150 to 250

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bar) and compressed liquefied petroleum gas (at 20 to 40 °C and 5 to 25 bar). Those authors found little differences in yields between both methods. The highest yield in $scCO_2$ was 12.62 wt%, at 40 °C ad 250 bar, whereas for PLE the highest yield was 12.07 wt%, at the same temperature and 25 bar. Furthermore, the PLE extraction showed good results in terms of solvent and time spent, decreasing the solvent/feed mass by a factor of 30 and the extraction time by a factor of 15.

Recently, Araújo [10] investigated the CXL extraction of oil from spent coffee grounds, and Juchen *et al.* [11] reported a study concerning SFE of rice bran lipids. Both applied a semi-batch process approach that provided similar yields as the traditional continuous process with lower solvent consumption.

Despite the different studies conducted for oil recovery from several raw materials, where the extraction efficiency and technical feasibility are demonstrated, the thermodynamic backgrounds and the kinetic aspects of this process are still in need of greater debate. Thus, this paper aims to present and discuss some aspects related to the extraction process using $scCO_2$ + organic solvents (ethanol specifically) in a semi-batch extraction system. For this purpose, two different study cases based on previous results obtained in our research group are used: spend coffee grounds [10] and rice bran [11] oil extraction in a semi-batch process approach.

## SEMI-BATCH METHODOLOGY OF SUPERCRITICAL $CO_2$+SOLVENT EXTRACTION

A laboratory scale unit used for the semi-batch extraction with $scCO_2$ + organic solvent (in the present case, ethanol is used) mixtures is presented in Figure 1. The experimental setup basically consists of a jacketed extractor vessel with a thermostatic bath for temperature control; a syringe pump for $CO_2$ injection and pressure control during the extraction; a needle valve for the flow control; and pressure and temperature transducers and indicators. Also, an extra thermostatic bath is used for temperature control in the syringe pump jacket. More details are about this extraction set up cab be easily found in the literature [10,11,16].

A typical extraction run starts with loading the raw material into the extraction vessel and, after that adding the liquid co-solvent in a fixed co-solvent to raw material mass ratio. After that, the vessel is closed, the extraction temperature is set and $CO_2$ is injected using the syringe pump up to the desired pressure condition for the extraction. At this moment, the first step of the extraction process begins, named static extraction period. Usually, a period of 10 min to 90 min (depending on the raw material) is used. This static extraction step is needed to ensure that the system has reach the equilibrium conditions, in terms of solvent percolation into the raw material, and both thermal and mechanical stabilization. After this period, the dynamic extraction step is started by opening the flow control valve; then the compressed $CO_2$ pass throughout the extraction bed containing the sample, ethanol and $CO_2$ loaded into the extraction vessel. Thus, it is essential to highlight that at the beginning of the dynamic extraction step the solvent system is like a CXL and, after the ethanol is completely extracted from the vessel the process shifts to a process with pure supercritical $CO_2$-like

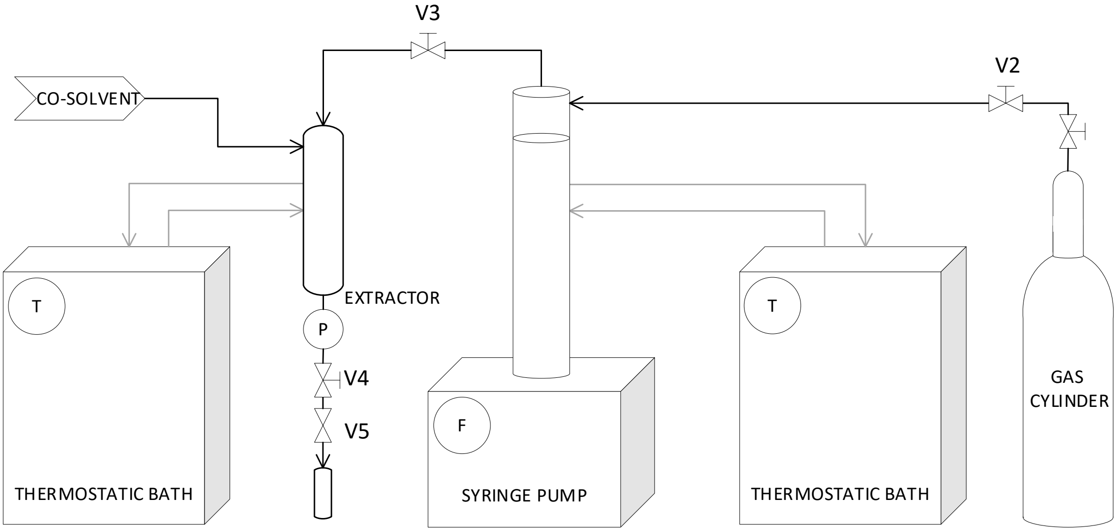

**Fig. 1.** Schematic diagram representing the laboratory scale unit used for semi-batch extraction using $scCO_2$ + ethanol mixture as solvent for oil extraction from waste raw materials.

extraction. Hence, because the amount of liquid solvent (ethanol) in the extraction vessel is fixed there is a switch of the solvent system during the extraction.

## DISCUSSION

In this section, two study cases are presented and discussed in terms of the overall performance of these extraction systems. The first case study is related to spent coffee grounds as presented by Araújo [10], and the second one is related to the rice bran oil extraction as presented by Juchen *et al.* [11]. Both authors evaluated the effects of the main process variables on oil extraction yield, such as pressure, temperature, confinement time and solvent to solids mass ratio. Also, both tested sequential extraction procedures in their works as an optimization strategy. After a brief presentation and discussion of the overall extraction curves of these two raw materials, some thermodynamic aspects considering the phase behavior and density of $CO_2$ + ethanol mixture are presented in relation to the conditions typically used in semi-batch $scCO_2$ + ethanol extraction procedure.

### *Case 1 – Oil extraction from spent coffee grounds*

Araújo (2019) [10] evaluated the extraction of spent coffee grounds at 40 to 80 °C, 10 to 20 MPa, using $scCO_2$ and ethanol as co-solvent in a semi-batch GXL extraction. For comparative purposes, the oil extraction schemes in PLE and SFE (with $scCO_2$ only) were tested as well.

Figure 2 presents the overall extraction curves of different temperature and pressure conditions using an ethanol to biomass ratio of 2:1.

Figure 2 depicts little difference in the initial extraction rates among the studied conditions. At the same temperature, the lowest extraction pressure was always faster reaching the equilibrium, indicating that a highly packed bed may increase the mass transfer resistance. At 80 °C, the maximum yield at 10 MPa was almost four points percents lower than at 20 MPa. This dramatic effect probably happened due to a phase split of the solvent mixture (see discussion in the next section), which is coherent with the phase diagram further presented in Figure 10.

With pure $scCO_2$, the maximum extraction yield was insignificant, suggesting that the oil inside spent coffee grounds is scarcely available, and as expected its solubility in pure $CO_2$ was very low. However, as the ethanol to biomass ratio increases, the maximum yield tends to approach the one acquired using pure ethanol in a continuous flow scheme (PLE).

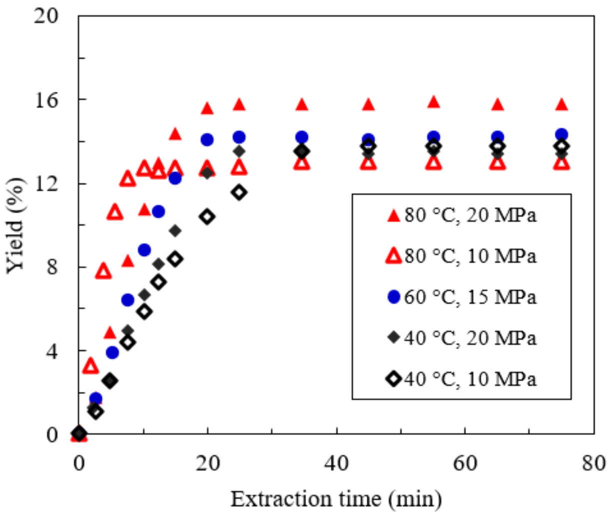

**Fig. 2.** Experimental overall extraction curves obtained by Araújo (2019) [10] for spent coffee grounds oil extraction with $scCO_2$+EtOH at ethanol to biomass ratio of 2:1.

Figure 3 depicts the kinetic curves reported by Araújo (2019) [10] at 60°C and 15 MPa with different co-solvent to biomass ratio and this revealed interesting trends from an optimization point of view. The initial extraction rate is higher at lower ethanol loadings, and the maximum yield is higher at higher ethanol loadings. Thus, sequential extractions using fresh ethanol injections can reduce the extraction time and both solvent and co-solvent consumptions to obtain the same final yield of a semi-batch CXL extraction.

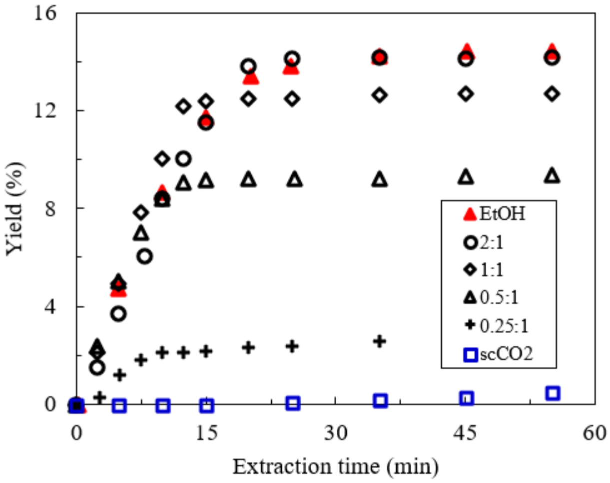

**Fig. 3.** Experimental overall extraction curves obtained by Araújo (2019) [10] for spent coffee grounds oil extraction with $scCO_2$+EtOH at different ethanol to biomass ratios in comparison to SFE with $scCO_2$ and PLE with ethanol.

Araújo (2019) [10] also evaluated a sequential extraction approach and the corresponding kinetic extraction curves are shown in Figure 4, in which, one can notice that the maximum yield obtained by sequential extraction, with ethanol to biomass ratio of 0.5 to 1 is analog to the pure ethanol, and to the one acquired by a regular extraction with ethanol to biomass ratio of 2:1.

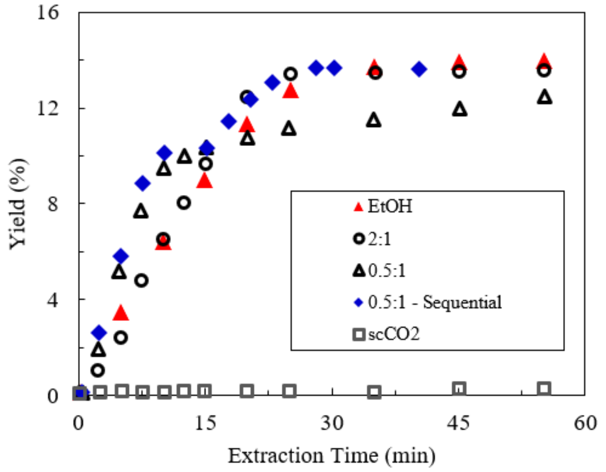

**Fig. 4.** Experimental overall extraction curves obtained by Araújo (2019) [10] for spent coffee grounds oil with scCO$_2$+EtOH at different ethanol to biomass ratios in comparison to SFE with scCO$_2$ and continuous PLE with ethanol, at 40 °C and 10 MPa. Comparison of one step with sequential extraction processes.

### *Case II – Oil extraction from rice bran*

Juchen et al. (2019) [11] studied the oil extraction from rice bran at the same temperature and pressure conditions (40 to 80 °C and 10 to 20 MPa), comparing pure scCO$_2$ with scCO$_2$ + ethanol as co-solvent in a semi-batch process, using ethanol to biomass ratios between 0:1 and 2:1.

The effect of pressure on maximum yield was positive, while the impact of increased extraction temperatures was highly negative on both extraction systems. By maintaining the temperature at 40 °C and the ethanol to biomass ratio at 1:1, the maximum yield increased from 16.26 % (10 MPa) to 25.48 % (20 MPa). On the other hand, maintaining the same ethanol to biomass ratio and the pressure at 10 MPa, the maximum yield decreased from 16.26 % to 2.60 % when temperature was increased from 40 to 80 ºC. This effect may have happened because up to 27 MPa, the CO$_2$ density controls the extraction process, while over 27 MPa the vapor pressure of the oil components dominates the extraction process [17].

Figure 5 present the extraction kinetics obtained at different ethanol to biomass ratios and PLE extraction carried out continuously with pure ethanol at the same conditions, while Figure 6 shows a sequential run with ethanol to biomass ratio of 1:1. All the experiments were performed at 40 ºC and 20 MPa.

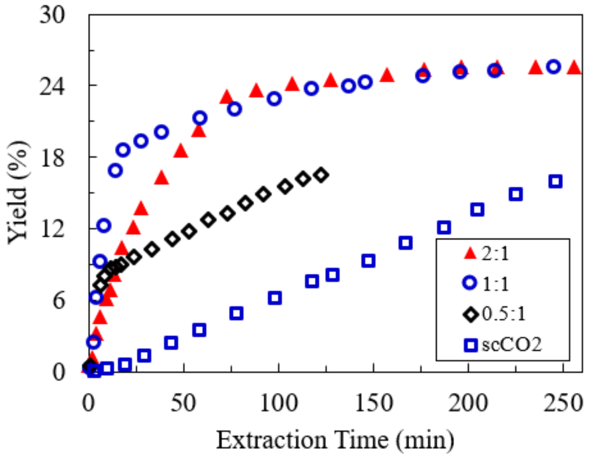

**Fig. 5.** Experimental overaal extractions curves obtained by Juchen et al. (2019) [11] for spent coffee grounds oil extraction with scCO$_2$+EtOH at different ethanol to biomass ratios in comparison to SFE with scCO$_2$ and continuous PLE with ethanol, at 40 °C and 20 MPa.

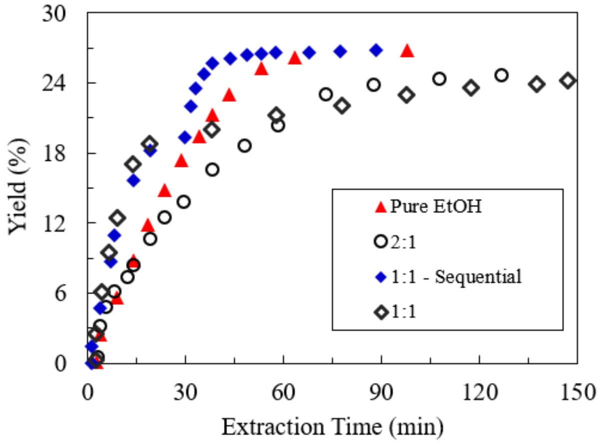

**Fig. 6.** Experimental overall extraction curves obtained by Juchen et al. (2019) [11] for rice bran oil extraction with scCO$_2$ + EtOH at an ethanol to biomass ratio 1:1 and 2:1 in comparison to continuous PLE with ethanol, at 40 °C and 20 MPa. Comparison of one step with sequential extraction processes.

Figure 5 exhibits that the pure scCO$_2$ extraction was technically viable for lipid extraction from rice bran due to its higher availability in comparison to lipids from the spent coffee grounds. Also, the addition of ethanol as co-solvent increased the extraction rate. By contrast, Figure 6 demonstrates that PLE and sequential extraction provided similar results. However, the first spent 21.09 g EtOH/g biomass and the second spent only 2.12 g EtOH/g biomass.

### *Some Thermodynamics Aspects*

Since the thermodynamic behavior of the solvent mixture plays a vital role in both SFE and CXL, it is convenient to analyze the thermodynamic diagrams in terms of pressure-composition and density-composition at the operational conditions of semi-batch CXL extraction processes. However, the thermodynamic analysis essentially lays on the reliability of thermodynamic the models. Cubic equations of state are simple and computationally efficient thermodynamic models that have been used to predict the phase behavior of different systems involving $CO_2$ and short chain alcohols and esters [18–20], although it is well known that these models do not properly represent the volumetric properties of the liquid phase (for mixtures or pure compounds). On the other hand, the Cubic Plus Association (CPA) equation of state [21], which keeps the physical parts of a cubic equation of state and adds the association term of Statistical Association Fluid Theory (SAFT) as proposed by Chapman et al. [22] providing a better tool for predicting both phase equilibria and density of associating mixtures without demanding high computational cost when compared to original SAFT-EoS. Thus, in this work the Peng-Robinson equation of state (PR-EoS) was compared to CPA for predicting both phase behavior and density of $CO_2$ + ethanol mixtures (the system of interest for the study cases presented here).

Figure 7 depicts pressure-composition diagrams for $CO_2$ + ethanol at two different temperatures. Figure 8 and Figure 9 show density-composition diagrams for the same mixture. For all these analyses, values predicted by PR-EoS and CPA-EoS were compared to the experimental data.

All diagrams calculated via PR-EoS considered the binary interaction parameters adjusted and presented by Araújo et al. [23]. The pure components parameters of CPA EoS used in this work were: ethanol ($a_0$ = 7.3110 bar.L$^2$.mol$^{-2}$, $b_0$ = 0.0479 L.mol$^{-1}$, $c_0$ = 0.9200, with one positive and one negative association site: $\varepsilon^{AiBi}$ = 207.64 bar.L.mol$^{-1}$ and $\beta^{AiBi}$ = 0.0160) and $CO_2$ ($a_0$ = 3.5256 bar.L$^2$.mol$^{-2}$, $b_0$ = 0.0271 L.mol$^{-1}$, $c_0$ = 0.7119, considered with two negative sites). Thus, $CO_2$ was considered non-self-associating but it able to make a cross association with ethanol due to the hydrogen bonds.

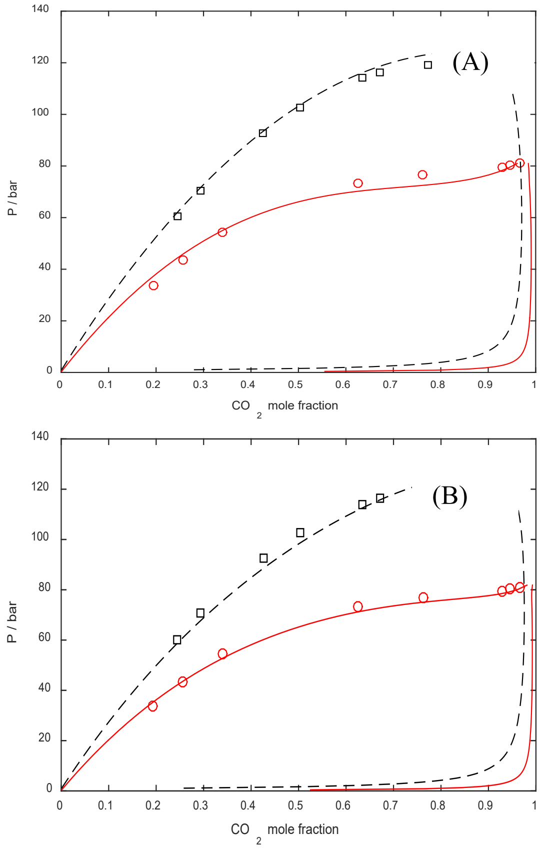

**Fig. .7**. Pressure-composition diagram for $CO_2(1)$ + ethanol(2) at 313 K (red circle) and 343 K (black square). Comparison between **(A)** PR-EoS ($k_{12}$ = 0.065, $l_{12}$ = -0.045) and **(B)** CPA ($k_{12}$ = 0.035, $l_{12}$ = -0.085, cross-association parameters: $\varepsilon^{AiBj} = \varepsilon^{Ai}/2$ and $\beta^{AiBj} = \beta^{Ai}$, where *Ai* represents the association site of self-associating compound) predictions and experimental values [24].

Figures 8 and 9 present the evolution of the mixture density when the $CO_2$ fraction increases and make clear that, even though both evaluated EoS were equivalent to reproduce the phase equilibrium data for density predictions the CPA-EoS showed to be more suitable for the studied system, as expected. It is also possible to notice that, at all evaluated conditions, there is a region that the mixture becomes denser than the pure ethanol as the $CO_2$ fraction increases. This occurs owing to the capacity of the liquid of comporting the gas phase. At higher $CO_2$ fractions, however, the mixture density tends to the density of the pure $CO_2$.

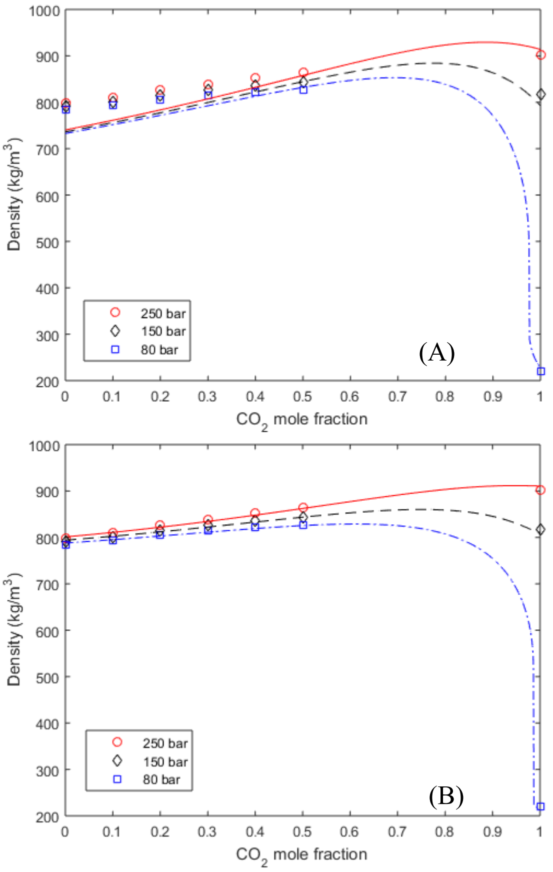

**Fig. 8.** Density-composition diagram for $CO_2$(1) + ethanol(2) at 308 K and different pressures. Comparison between **(A)** PR-EoS ($k_{12}$ = 0.065, $l_{12}$ = -0.045) and **(B)** CPA ($k_{12}$ = 0.035, $l_{12}$ = -0.085, cross-association parameters: $\varepsilon^{AiBj} = \varepsilon^{Ai}/2$ and $\beta^{AiBj} = \beta^{Ai}$, where $Ai$ represents the association site of self-associating compound) predictions and experimental values (Ref1. [25]). Pure ethanol and $CO_2$ were obtained from (Ref. 3 [26,27]) and (Ref. 2 [28]), respectively.

Finally, in Figure 10, one can notice the extraction pathway going from the most concentrated in ethanol condition to the condition where there is only $CO_2$ (right side). At lower pressures, below 150 bar, may occur phase partition of the mixture, which is undesirable to extraction processes due to additional mass transfer resistance among phases and the reduced particle contact with one of the solvents since the vapor phase is high concentrated in $CO_2$. Also, the vapor phase density is much lesser dense than the liquid phase and presenting much lower solvent power when compared to the respective liquid phase in equilibrium. Therefore, the knowledge of phase envelops of the mixtures supercritical $CO_2$ plus the organic solvent used for semi-batch extraction at high pressure conditions is an important issue and it must be considered for setting the operational conditions for this technique.

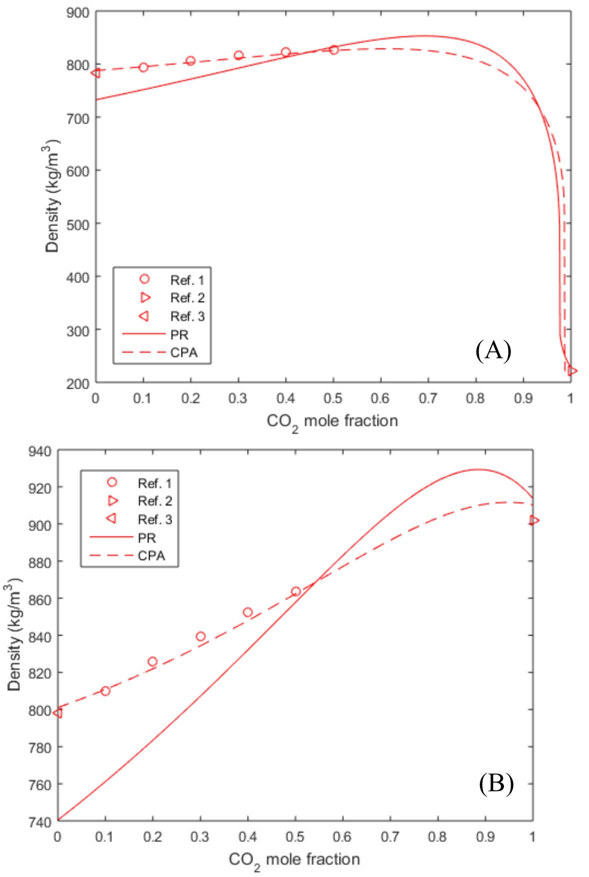

**Fig. 9.** Density-composition diagrams at 308 K and different pressures: 70 bar **(A)** and 250 bar **(B)** comparing both PR and CPA models. Using the following parameters: PR-EoS ($k_{12}$ = 0.065, $l_{12}$ = -0.045) and CPA ($k_{12}$ = 0.035, $l_{12}$ = -0.085, cross-association parameters: $\varepsilon^{AiBj} = \varepsilon^{Ai}/2$ and $\beta^{AiBj} = \beta^{Ai}$, where $Ai$ represents the association site of self-associating compound) predictions and experimental values (Ref1. [25]). Pure ethanol and $CO_2$ were obtained from (Ref. 3 [26,27]) and (Ref. 2 [28]), respectively.

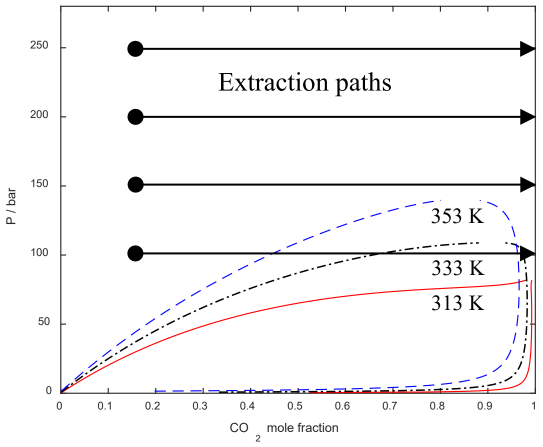

**Fig. 10.** Projection of extraction paths in a pressure-composition diagram for the $CO_2$ + ethanol system, at typical extraction conditions of temperature (313 to 353 K) and pressure (100 to 250 bar). Lines represent the phase envelops predicted using CPA EoS.

## CONCLUSION

This study highlighted and discussed some kinetic and thermodynamic aspects of results previously reported by the Research Group through a semi-batch GXL extraction strategy of rice bran and spent coffee grounds oil. Even though the matrices presented different structural characteristics, with the rice bran oil much more available than the spent coffee grounds oil, the semi-batch GXL extraction was shown as a promising extraction process, primarily when sequential extractions are performed. The thermodynamic behavior of the solvent mixture was debated at different extraction conditions, and some trends observed on the kinetic curves could be justified and predicted through this type of analysis. Additionally, it was demonstrated that CPA-EoS has a better prediction capacity for the density of the studied systems than the PR-EoS.

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# Studies on extraction from Avocado's waste biomass to generate process design alternatives of valuable products

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Avocado (*Persea americana*) seeds of the cultivar Hass, cultivated in Brazil and Mexico, were study for extraction purposes, in order to determine the amount and the composition of valuable extracts. The avocado seeds, which represent about 23 % of fruit weight, have even higher antioxidant activity than its pulp. This study carried out the extraction of seed oil by using two extraction techniques: Soxhlet liquid-solvent extraction and supercritical fluid extraction (SFE). Soxhlet extraction (for a period of 6 hours) involved three solvents: hexane, ethanol and ethyl acetate. Ethanol presented the highest extraction yield, for both particle sizes studied and for both seed types (Mexican and Brazilian), being the highest yield of 10.3 ± 0.3 % for the smaller particles, referring to the Brazilian seeds. This result indicated that the seed's extract might be high on content for polar components. The SFE was performed using supercritical carbon dioxide ($scCO_2$) as a solvent and after some preliminary experiments, ethanol revealed to be the best cosolvent. The highest extraction yield (for particle size between 0.42 and 0.60 mm) was 6.9 % for the conditions of 80 ºC, 25 MPa and a mass ratio of 1.5:1 (referring once again to the Brazilian seed). Comparing both methods, Soxhlet reached higher yield, although it implies a greater energy, time and solvent consumption than SFE.

**Key words:** Avocado seed, Supercritical fluid extraction, Soxhlet extraction, yield, ethanol

## INTRODUCTION

The transformation of waste biomass into valuable materials and/or energy is emerging as a powerful trend due to the depletion of natural resources, increased greenhouse emissions and awareness of the need for sustainable development in terms of safe reuse of waste and biomass [1].

A substantial quantity of biological waste (such as fruit's peels and seeds) is produced due to significant consumption and industrial processing of the edible components of the plants. One of the most useful strategies is to recover bioactive compounds, particularly phenolic compounds, making complete use of them in the food, pharmaceutical and cosmetics industries [1]. In this study, the waste biomass was Hass avocado's seeds from Mexico and Brazil, as only the avocado pulp is used for commercial purposes, while its peels and seeds are discarded. Avocado's seeds are known for its high content of phenolic compounds and high antioxidant capacity, as well as for being rich in essential fatty acids such as linoleic and linolenic acid, which are beneficial to human cardiovascular health [2].

Most biomass waste is a complicated and variable molecular combination, and separation is the main problem. Moreover, usually some of the bio-waste and the separating materials are solid, therefore, organic solvents are often involved in separation. In order to make bio-based chemical production self-sustainable, these solvents must also be bio-based and cannot be obtained from crude oil in the long term [1]. For that, it is essential to reduce extraction methods that recur to organic solvents like Soxhlet extraction. So, a more environment-friendly method is the Supercritical Fluid Extraction (SFE), in which carbon dioxide ($CO_2$) acts as a non-toxical and non-flammable solvent. Supercritical $CO_2$ ($scCO_2$) is regarded as the most common supercritical fluid due to the moderately critical temperature and pressure (31.1ºC and 7.4MPa), Its density and solvability are intermediate between gas and liquid and can be readily altered with small modifications in temperature and pressure. In addition, by merely reducing the pressure, it is totally isolated from the extract, thus achieving a product with a high degree of purity that is not feasible when using organic solvents [3-6].

This study addresses the avocado's seed usage to obtain valuable extract by using different extraction techniques: SFE (mainly $CO_2$ + ethanol) and Soxhlet extraction (hexane, ethanol and ethyl acetate). The extraction yield and the solvents selectivity used is compared in both e extraction techniques. The particle size was also evaluated in the Soxhlet method.

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## EXPERIMENTAL

### *Sample collection and pre-treatment*

Avocado fruits from Mexico and Brazil, both from the cultivar Hass, were purchased from the local market, although the Mexican avocados were bought at a Portuguese market and the Brazilian avocados were bought at a market situated in Brazil. The fruits were stored at room temperature (approximately 22ºC) until they become ripe. The avocados (free from any apparent skin damage) were then washed with tap water (to remove any remaining dirt), cut open with a knife and the seeds, as well as the peels and pulp, were manually removed. The pulp was discarded, the peels were stored, and the seeds were cut manually into smaller dimensions, subsequently being oven dried with air circulation (Solab, model SL-100, Brazil) for 24 hours at 55 ± 1 ºC. Afterwards, the seeds were milled in a coffee and meat mill (Arbel, Brazil) in order to be classified, according to particle size, using a system of Tyler series sieves (Bertel, Indústria metalúrgica Ltda, Brazil). The series of sieves chosen were 9, 12, 24, 28 and 35 meshes and they were put through a vertical vibratory sieve shaker to improve particle size classification.

The seeds retained between the 28 and 35-mesh and below 35-mesh (#28 = 0.60 mm and #35 = 0.42 mm) sieves were chosen for extractions purposes, once those meshes yielded great quantity after milling. However, only the particles from 28 and 35-mesh will be used in supercritical fluid extraction. The milled samples were packaged in vacuum polyethylene bags and stored at -18ºC until required for analysis.

Seed moisture on a dry basis (45.45 ± 1.69 wt%) was determined by the gravimetric method which consists of drying the sample in the oven at 105 ± 1ºC until constant mass is obtained, as it is calculated by the loss of weight upon the dry sample.

### *Chemical and reagents*

All the solvents used were of analytical grade.

For the conventional extraction method (Soxhlet extraction) hexane (99 % purity, Êxodo Científica, Brazil), ethanol (99.8 % purity, Neon, Brazil) and ethyl acetate (99.5 % purity, Neon, Brazil) were used. For the supercritical fluid extractions, it was used $scCO_2$ (99.5 % purity in the liquid phase, White Martins Gases Industriais Ltda, Brazil) as the main solvent. As cosolvents for these extractions, ethanol (99.8 % purity, Neon, Brazil) and ethyl acetate (99.5 % purity, Neon, Brazil) were used.

### *Soxhlet extraction*

The extraction was held with a Soxhlet apparatus (Uniglas, Brazil) constituted by the Soxhlet condenser and extractor, in which approximately 5 g of avocado's dry seeds (Brazilian or Mexican) were transferred to a handmade filter paper bag (6.5 x 4.5 cm) and placed inside the Soxhlet extractor apparatus of 250 ml. Afterwards, a pure solvent (hexane, ethanol or ethyl acetate) was added inside the extractor, for about 180 ml, into a flask of 250 ml attached to the apparatus and heated up beyond its respective boiling point, in order to achieve a constant condensation rate. The experiments were carried out by a period of 6 h (confirmed by the kinetic study) with an average cycle time of 20 ± 3 min.

At the end of the extraction, the solvent was removed in vacuum using a rotary evaporator (RV 10 digital, IKA, Wilmington, USA) at approximately 40ºC and 30 rpm to recover the seed oil. The flask containing the oil was then kept in an oven with air circulation at 60 ± 1 ºC to guarantee full removal of residual solvent for about 48 h. Following the drying and after cooling to room temperature, the sample was weighed to determine the extraction yield, transferred into a 2 ml vial and stored under refrigeration (-18 ºC) for chemical analysis.

The extraction runs were carried out in triplicate at identical conditions and mean values were used.

The extraction yield was expressed as a mass per cent of the extracted oil in relation to the initial mass of the seeds used for extraction according to Eq. (1):

$$Yield\ (\%) = \frac{mass\ of\ extracted\ oil}{mass\ of\ avocado's\ seed\ sample} \times 100 \qquad (1)$$

### *Supercritical fluid extraction (SFE)*

The extractions using $scCO_2$ as a solvent were performed in a bench scale unit (Fig. 1), which has been described in previous studies [3-5].

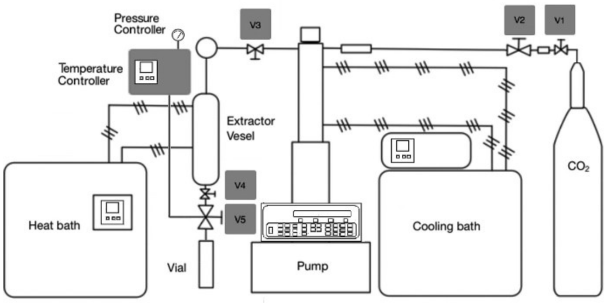

**Fig.1.** Schematic diagram of the extraction unit. V1 and V2: cylinder valve; V3: ball valves; V4: gas pressure regulator valve; V5: micrometric valve (adapted from [3]).

Briefly, the experimental setup consists of a stainless steel vessel extractor, with a volume of 65 cm$^3$, a diameter of 1.9 cm and a height of 22 cm, coupled to a heating thermostatic bath (Quimis, Model Q214S2, Brazil), a micrometric valve to control the CO2 flow rate, a syringe-type pump controller (Teledyne ISCO 500D, USA), which temperature was maintained at 10 °C for all experiments using another thermostatic bath (HipperQuímica, Brazil), temperature controller (NOVUS Produtos Eletrônicos Ltda, Brazil) and pressure controller (WIKA, Brazil).

Besides $CO_2$, ethanol was used as a cosolvent for the extraction, in order to increase both extractions yield and rates, since it was the most efficient cosolvent evaluated in previous tests (compared to ethyl acetate). Therefore, these extractions were named $scCO_2$+EtOH.

The procedure of the extraction with $scCO_2$+EtOH consisted of placing around 10 g of avocado's seed (Brazilian or Mexican) soaked (wetted) with ethanol, inside the extractor, at the desired ethanol to raw material mass ratio. This being said, for 10 g of avocado's seed, the amount of ethanol added was: 10 g (mass ratio of 1:1), 15 g (mass ratio of 1.5:1) and 20 g (mass ratio of 2:1), although the latter was only tested at the centre point.

Then the extractor was filled with $CO_2$ by injection, the pressure and temperature were set at the desired conditions and the static extraction begun for a certain confinement period (30 min using cosolvent).

After the end of static extraction, the dynamic extraction started by using compressed $CO_2$ at a constant flow rate around 2.0 ml/min, which was used for all extractions of this study and it was controlled by the syringe-type pump at cooling temperature and the pressure of the extractor.

The oil extracts were collected in test tubes, immediately covered with their respective taps, at 2.5 min (up to 25 min), 5 min (25-30 min) and 10 min (30-60 min).

At last, after sampling, the test tubes were placed inside an air circulating oven, in order to evaporate the ethanol until constant mass is achieved (for about 48 h) and then the dried extracts were gravimetrically quantified for each sampling and stored at −18 °C.

To determine the conditions of pressure and temperature of these experiments, a Design of Experiments (DoE) was applied (Table 1). In this study, a $2^2$ full factorial experimental design with duplicate on centre points was used to analyse the impact of the independent variables: temperature (40, 60 and 80 ºC) and pressure on the extraction yield (15, 20 and 25 MPa), on both mass ratios (1:1 and 1.5:1).

The influence of density, which depends on the temperature as well as pressure, has also been evaluated. The $CO_2$ density values were obtained from the NIST database [7] at the temperature from the cooling bath (10 ºC) and the system's pressure (15, 20 and 25 MPa).

The oil extraction yield was calculated according to Eq. (1).

## RESULTS AND DISCUSSION

### *Soxhlet extraction*

The purpose of both extractions' method (Soxhlet or SFE) is to evaluate and compare the extraction yield as well as comparing it between both samples (Mexican and Brazilian).

Soxhlet extraction was carried out in triplicate for two different particles sizes, using different Tyler's meshes. One size consisted of the particles retained from mesh 28 and 35 (0.60 mm > particle > 0.42 mm) and the other size was representative of the particles who were retained below mesh 35 (particle < 0.42 mm).

In all extractions, of both particles' sizes, three organic solvents were used: hexane, ethanol and ethyl acetate. The choice of the solvent to be used depends on the substances that are going to be extracted from the samples since the polarity of the solvent has high importance. In this study, for future purposes, polyphenol compounds are primarily of a polar type, polar solvents such as ethanol, ethyl acetate can effectively extract them. Still, hexane is well known for its efficiency in oil extraction, which may improve future extraction of fatty acids, since they are both non-polar [2-6].

**Table 1.** Results of oil extraction yields of avocado's seed using Soxhlet with hexane, ethanol and ethyl acetate

| Run | Solvent | T(°C) | PS (mm) | Extraction yield (wt%) |
|---|---|---|---|---|
| Mexican seed | | | | |
| 1 | Hexane | 68.00 | [0.42; 0.60] | 2.46 ± 0.03 |
| 2 | EtOH | 78.38 | [0.42; 0.60] | 8.2 ± 0.9 |
| 3 | EtOAc | 77.10 | [0.42; 0.60] | 3.6 ± 0.1 |
| 4 | Hexane | 68.00 | < 0.42 | 3.27 ± 0.02 |
| 5 | EtOH | 78.38 | < 0.42 | 9.8 ± 1.9 |
| 6 | EtOAc | 77.10 | < 0.42 | 3.1 ± 0.3 |
| Brazilian seed | | | | |
| 7 | Hexane | 68.00 | [0.42; 0.60] | 3.5 ± 0.1 |
| 8 | EtOH | 78.38 | [0.42; 0.60] | 9.5 ± 0.2 |
| 9 | EtOAc | 77.10 | [0.42; 0.60] | 4.6 ± 0.2 |
| 10 | Hexane | 68.00 | < 0.42 | 3.6 ± 0.1 |
| 11 | EtOH | 78.38 | < 0.42 | 10.3 ± 0.3 |
| 12 | EtOAc | 77.10 | < 0.42 | 4.8 ± 0.4 |

Table 1 presents the results of Soxhlet extraction using hexane, ethanol and ethyl acetate as solvents for different particle sizes (PS), for 6 hours of extraction.

As shown in Table 1, ethanol provided higher extraction yields when compared to hexane and ethyl acetate for all particle sizes (PS) evaluated, either Mexican or Brazilian, being the highest yield referring to the Brazilian seed with the particle size being lower than 0.42 mm.

In terms of the origin of the avocado's seed, although both samples are from the same variety (Hass), the ones originated from Brazil had always a higher yield than the ones who came from Mexico, in both particles sizes (Fig.2). This difference might have to do with the composition of avocado or any other fruit being dependent on the variety, grade of ripening, climate, the composition of soil and fertilizers [2].

In terms of particles sizes, the effectiveness of all solvents was considerably improved by lower particle diameters, providing higher oil yields, except in the Mexican seed, using ethyl acetate, in which the lower particle diameter had less oil yield. However, the authors decided that the raw material with lower particle size would be used only for comparison, because for further experiments with SFE, the solid matrix should be intermediate, as over-milling can lead to too fine particles, limiting fixed-beds performance, caused by the agglomeration of particles, dead zones formation and compaction [8].

The comparison of experimental results with the ones available from the literature [9-11] can be seen in Fig.2, using the average extraction yield of both particles sizes, divided by the hours of extractions.

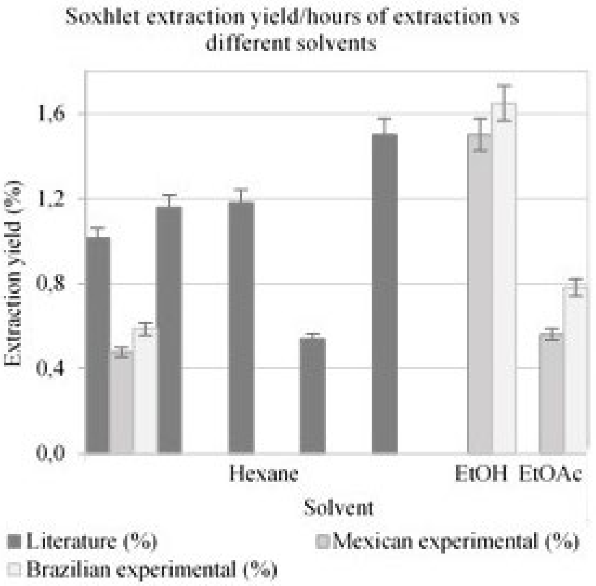

**Fig.2**. Experimental results from Soxhlet vs literature

Analyzing Fig.2, it was not found literature results for Soxhlet extraction using ethanol or ethyl acetate for avocado's seed, as far as the authors know. Despite that, literature results were found using hexane as a solvent for avocado's seed [9-11]. As it can be seen, taking into consideration the hours of the extraction of all experiments, most of the literature's extraction yield was higher than the ones obtained from this study with hexane, although when using ethanol, these results were similar. However, the varieties or the origins of the avocado's seed from the literature are not the same as the avocado's variety studied, which can explain the differences between yields.

### *SFE*

Before a full DoE was applied, it was performed some preliminary experiments with the aim to find what cosolvent would help achieve the highest yield, in the Brazilian sample, since it had a higher yield than the Mexican sample, and also there was more quantity of it (with particle size from mesh 28 and 35 only). Since ethanol and ethyl acetate had better results as solvents in the Soxhlet extraction, they were tested in SFE. These experiments showed that pure scCO2 was not efficient enough by itself to extract the seed oil, at conditions up to 20 MPa and 60 °C (extraction yield around 3%), for 60 minutes of confinement time, being needed the addition of ethanol or ethyl acetate to optimize the extraction. Yet, ethanol obtained better results of extraction yield than ethyl acetate, being the cosolvent chosen for the full DoE (Table 2).

As presented in Table 2, the experiments are referred to the Brazilian sample. Analyzing the results obtained, the higher yields were achieved at higher pressure (25 MPa) and temperatures of 40 and 80ºC, being the highest yield of 6.9% for the mass ratio of 1.5:1 (run 20). Contrarily, the lowest yield was 1.9% for the mass ratio of 1:1, at 15 MPa and 40ºC (run 16).

These results are directly influenced by $CO_2$ density, in which higher density increases the extraction yield by improving $CO_2$ solubility power into the matrix [3,4].

For the Mexican sample, no preliminary experiments were performed, but full DoE with the mass ratios of 1:1 and 1.5:1 experiment was (table 3). The highest extraction yield was 4.4% at 15 MPa and 80 ºC (run 32) for mass ratio 1.5:1 and the lowest yield was 1.6% at 15 MPa and 40 ºC (run 33) for mass ratio 1.5:1. Contrarily to the results of the Brazilian sample, the Mexican seed had its highest yield at a lower pressure which means lower density, and for that, we can say that the temperature of extraction had a more significant effect on the yield than the pressure.

Considering Tables 2 and 3, Soxhlet extraction with ethanol is considered as a value of reference for

**Table 2**. Experimental conditions and results for Brazilian avocado's seed oil extraction yields for SFE using $scCO_2$ and cosolvents

| Run | Condition | Pressure (MPa) | Temperature (°C) | $CO_2$ density (kg/m$^3$) | Extraction yield (wt%) |
|---|---|---|---|---|---|
| *Soxhlet* | | | | | |
| 7 | Hexane | | 68.00 | | 3.5 ± 0.1 |
| 8 | EtOH | | 78.38 | | 9.5 ± 0.2 |
| 9 | EtOAc | | 77.10 | | 4.6 ± 0.2 |
| *SFE - preliminary experiments* | | | | | |
| 10 | $CO_2$ | 25 (+1) | 40 (-1) | 1001.7 | 2.7 |
| 11 | $CO_2$ + EtOAc | 25 (+1) | 40 (-1) | 1001.7 | 2.6 |
| 12 | $CO_2$ + EtOH | 25 (+1) | 40 (-1) | 1001.7 | 4.2 |
| 17 | $CO_2$ + EtOH (1.5:1) | 20 (0) | 60 (0) | 980.2 | 5.4 |
| 18 | $CO_2$ + EtOH (2:1) | 20 (0) | 60 (0) | 980.2 | 5.1 |
| *SFE - experimental design (1:1)* | | | | | |
| 13 | $CO_2$ + EtOH | 20 (0) | 60 (0) | 980.2 | 4.4 ± 0.5 |
| 14 | $CO_2$ + EtOH | 25 (+1) | 80 (+1) | 1001.7 | 6.3 |
| 15 | $CO_2$ + EtOH | 15 (-1) | 80 (+1) | 954.2 | 2.7 |
| 16 | $CO_2$ + EtOH | 15 (-1) | 40 (-1) | 954.2 | 1.9 |
| *SFE - experimental design (1.5:1)* | | | | | |
| 19 | $CO_2$ + EtOH | 20 (0) | 60 (0) | 980.2 | 5.0 ± 0.4 |
| 20 | $CO_2$ + EtOH | 25 (+1) | 80 (+1) | 1001.7 | 6.9 |
| 21 | $CO_2$ + EtOH | 25 (+1) | 40 (-1) | 1001.7 | 5.6 |
| 22 | $CO_2$ + EtOH | 15 (-1) | 80 (+1) | 954.2 | 5.0 |
| 23 | $CO_2$ + EtOH | 15 (-1) | 40 (-1) | 954.2 | 4.6 |

**Table 3**. Experimental conditions and results for Mexican avocado's seed oil extraction yields for SFE using $scCO_2$ and cosolvent

| Run | Condition | Pressure (MPa) | Temperature (°C) | $CO_2$ density (kg/m$^3$) | Extraction yield (wt%) |
|---|---|---|---|---|---|
| *Soxhlet* | | | | | |
| 7 | Hexane | | 68.00 | | 2.46 ± 0.03 |
| 8 | EtOH | | 78.38 | | 8.2 ± 0.9 |
| 9 | EtOAc | | 77.10 | | 3.6 ± 0.1 |
| *SFE - experimental design (1:1)* | | | | | |
| 24 | $CO_2$ + EtOH | 20 (0) | 60 (0) | 980.2 | 2.5 ± 0.3 |
| 25 | $CO_2$ + EtOH | 25 (+1) | 40 (-1) | 1001.7 | 2.4 |
| 26 | $CO_2$ + EtOH | 25 (+1) | 80 (+1) | 1001.7 | 3.1 |
| 27 | $CO_2$ + EtOH | 15 (-1) | 80 (+1) | 954.2 | 2.7 |
| 28 | $CO_2$ + EtOH | 15 (-1) | 40 (-1) | 954.2 | 2.0 |
| *SFE - experimental design (1.5:1)* | | | | | |
| 29 | $CO_2$ + EtOH | 20 (0) | 60 (0) | 980.2 | 3.7 ± 0.6 |
| 30 | $CO_2$ + EtOH | 25 (+1) | 80 (+1) | 1001.7 | 3.9 |
| 31 | $CO_2$ + EtOH | 25 (+1) | 40 (-1) | 1001.7 | 3.6 |
| 32 | $CO_2$ + EtOH | 15 (-1) | 80 (+1) | 954.2 | 4.4 |
| 33 | $CO_2$ + EtOH | 15 (-1) | 40 (-1) | 954.2 | 1.6 |

its highest yield and for being a green solvent, with the right polarity for extracting a wide range of valuable compounds from the avocado's seed [3-5].

Therefore, the run from SFE that got closer to this value was run 20 from the Brazilian sample and run 32 from the Mexican sample, although the Mexican sample always presented lower yields than the Brazilian sample, for all SFE experiments, just like in the Soxhlet extraction. One reason for that is the fact the Brazilian sample was more porous than the Mexican sample and interacted strongly with the cosolvent added, consequently achieving higher yields.

Comparing both methods, Soxhlet extraction is known for its high extraction yields, justified by the fact it resorts to high temperatures that reduce the surface tension and viscosity of the solvent, improving its solubilization and increasing the number of the components soluble in the solvent phase. SFE extraction yields are lower than the results obtained by Soxhlet extraction, but still, it needs to be mentioned that the amount of solvent and energy spent in Soxhlet is much higher than the one spent in SFE (being harmful to the environment), in addition to spending much more time on the extraction than in SFE [2-6].

## CONCLUSION

In order to value avocado's by-products, namely the extracts from its seeds, extraction using $scCO_2$ and Soxhlet were studied, using different solvents.

The seeds that obtained the best results were the Brazilian seeds. For them, the highest yield was obtained by using ethanol as a solvent in the Soxhlet extractor for 6 hours (9.5 %). For the SFE extraction, the addition of cosolvent, in this case, ethanol as well, did increase the seed oil recovery (6.9 % for the highest value, at 80 ºC, 25 MPa and mass ratio of 1.5:1) compared to extraction using only $scCO_2$ (2.7 % at 60 ºC and 20 MPa), since it interacts with the polar fraction of the extract that the $scCO_2$ can not, for it is non-polar.

Even though the SFE method had lower yields results, it is of great importance because $CO_2$ is a more selective solvent than the organic ones.

For the Soxhlet method, different solvents were used, being the value for ethanol more than twice as high as for hexane, which confirms the role that polarity has on the extraction yield, indicating that the extract is richer in polar analytes than in non-polar. Size of particles was also a parameter evaluated, in which the smaller sizes enhanced the extraction yield (10.3 % for the Brazilian seed) by increasing the surface area in contact with the solvent.

Relatively to its advantages, SFE is more economical and environmentally friendly due to less use of solvent, energy and time of extraction, when compared to Soxhlet.

The results are useful for the further development of methods to extract bioactive compounds, in order to analyse the pharmaceutical application of these extracts.

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# *Opuntia ficus indica* (Prickly pear): extraction and characterization of products with anti-age and antioxidant activity

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The use and application of natural products in Food and Pharmaceutical industry is of great interest, since the beginning of human history and are the basis of modern medicine. In the present study the ethanolic extraction of *Opuntia ficus indica* is investigated. This plant has been used in traditional medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects [1]. Specifically, in this study, the plants were collected and split in two parts. One was consisted of the seeds and the other of the leaves and fruits. Subsequently, was held dry of the different parts of the plant. The method of the extraction process involved maceration followed by percolation and in the end of the process a strict protocol of quality control was applied as also an HPLC analysis. The extracts obtained via the specific extraction can be used either as individual medicinal formulations or in combination with other natural extracts to reestablish the healthy natural functions of the body. The current study, aims to production of pharmaceutical products such as capsules, tablets, drops, powder and elixirs from *Opuntia ficus indica*. The most important feature of these productions that all materials and products are non-toxic, natural and friendly to the environment.

**Key words:** prickly pear, extraction, antioxidant properties,

## INTRODUCTION

*Opuntia,* commonly called prickly pear [2] or nopal fruit, is a genus in cactus family, Cactaceae [3]. Prickly pear is native to North America but it has spread to Central South America, North Africa, Europe, Mediterranean countries, the Middle East, and other countries [4].

The fruit has a unique composition of nutrients, including high levels of vitamin C, B-family vitamins (B2, B3, B6, B9), minerals (Ca, Fe, Mg, P, K, Zn), and soluble fibers, and it can be consumed raw or dried. According to The American Journal of Clinical Nutrition (2004), prickly pears have high levels of flavonoids, polyphenols, and betalains[5]. All the parts (pips, skin, pulp, leaves) of prickly pear are exploitable and have anti-oxidant, anti-inflammatory and anti-aging properties.

The health benefits of prickly pear include its ability to lower cholesterol levels, improve the digestive process, decrease the risk of diabetes, and boost the immune system. The antioxidant-rich fruit also helps strengthen blood vessels, aid in weight loss, reduce inflammation and also protect the skin and lower the chances of premature aging [5].

In this work the medicinal properties of prickly pear are presented; methods of valuable compounds extraction are shown and the products of extraction are compared; extracts characterization and product quality control is demonstrated. Also, the potential uses of valorization of prickly pear fruit waste are referred.

Prickly pear has largely been ignored in the United States as a potential health-improving plant. Although demand for *Opuntia* as a vegetable for its fruit products is increasing (as the Hispanic population increases), its current economic value as a crop is much less than it might be, if produced for the manufacture of products used in treating hypoglycemia, diabetes, high blood cholesterol and obesity [6].

The fruit is rich in flavonoids, polyphenols and betalains that act as antioxidant compounds and neutralize free radicals before they cause healthy cells to mutate. Free radicals are partially responsible for the oxidation of neural cells that lead to various diseases. Polyphenolic compounds also, have been linked to increased cognitive activity [5].On the other hand, betalains are water-soluble nitrogen-containing pigments that are responsible for the bright red or yellow color of fruits, flowers, roots and leaves of plants. Prickly pear contains, particularly, betacyanins in the purple variety and betaxanthins in the orange variety [7].

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## MATERIALS AND METHODS

The prickly pear fruits were obtained from Greece and they were grown on the most suitable soil. Only natural fertilization was employed. Special attention was paid to ensure absence of any chemical fertilizers, pesticides, insecticides, fungicides, or herbicides.

The harvest of the plant (leaf together with fruit) was conducted according to a strict protocol and collection rules. The collected parts included unripe fruits, leaves and ripe fruits (in their early ripening stage-not fully matured). The collection took place during late mornings of August; the most suitable hour for its collection is late in the morning, to avoid moisture. Only the completely dry and clean (of parasites, or illness) fruits of the plant were collected. Meticulous cleaning of all kinds of impurities (organic or inorganic) followed. Only well-ventilated packages (perforated bags, baskets of reeds, boxes full of holes etc.) were used in order to rule out the growth of harmful enzymes and fungi. Furthermore, the longest length of transport of the plant never exceeded 48 hours.

### *Drying process.*

The leaves at the beginning went through a process called stabilization, whereby the leaves were steamed in 60% pure ethanol. Subsequently, the leaves had the thorns removed with special tools, and then were put in a well ventilated place in the shade for drying. Drying usually lasted 7-15 days (depending on the weather).

The thorns of the fruits were removed with a special brush; the fruits were then peeled and the peel removed was also placed for drying under the same conditions as the leaves.

Fruit seeds were separated from the unripen fruits. They were then subjected to a stabilization process. They were subsequently placed in the sun to dry 1–2 days prior to being used for the extraction. The stabilization process for natural entities which are intended to be used in an extraction process after being dried, involves the following: placement of the respective compounds in a form of a very thin layer on the top of a sieve tray; a very fine droplet spraying of the herbal substance with pure ethanol follows, so that the whole exterior surface of it is wetted. The ethanol is then left to dry. This process is the most suitable natural method of elimination of any type of fungi or bacteria which can affect the herbal entities and prevent its decay until it is taken for the extraction

### *Extraction.*

Three extractions were made, one of the leaves together with the peel of the fruits, one of the ripe fruits containing their seeds and one of the seeds of the unripen fruits.

The extraction process involved maceration followed by percolation

A. Maceration

The herbal substance had first been crushed to form a coarse powder. The resulting coarse powder (of a size appox5 mm) was mixed with the solvent, which was usually water, pure alcohol or a mixture of pure alcohol & water in a volume proportion 50:50. It was allowed to soak for 1day. During the day the mixture was shaken regularly & finally formed a pulp like extract.

B. Percolation

The aforementioned pulp containing the herbal substance, after the maceration, was placed in a special apparatus for 3 days with enough pure solvent (ethanol) to completely cover the herbal substance. Subsequently, it was placed inside the extractor a specific gravity with specific diameter and kg, according to specifications and it was kept so for at least 24 hours. The extract was filtered or it was left to rest until it was clear.

**Fig.1**. *Opuntia ficus indica* extracts after maceration and percolation process; from left to right: extracts from unripe fruit seeds (in conical flask), from leaves and fruit peels (large beaker with light colored liquid), and from ripe fruits containing their seeds (smaller size beaker), respectively.

*HPLC analysis.*

The samples were analyzed using a Luna, 4.6×150 mm, 5 m particle size, 100 °A pore size column. A 40:60 acetonitrile:water solution was used for 14 min, as an isocratic mobile phase at 0.5 ml/min flow rate and 30 ºC. The injection volume was 20 microliter and detection was through a UV detector set at 280 nm.

Well defined in a strict protocol quality controls were performed during the procedure and at the end of the process. Those protocols involve a number of analytical methods which have been developed in our laboratory through over 20 years of experience; they were based on traditional analytical methods of Galenea and French pharmacology but PANAX has advanced and specialized their applicability and reliability. The final quality control of the extract included determination of alcoholic degree and degree of acidity (pH) of the extracts.

## RESULTS AND DISCUSSION

In this work the treatment of prickly pear employed dried fruits to form powder and liquid extracts. They lead to production of pharmaceutical products such as capsules, tablets, drops, powder and elixirs. The most important feature of this production is that all materials and products are non-toxic, natural and friendly to the environment.

The results from the HPLC analysis from the three extractions are observed at the figures 2,3 and 4.

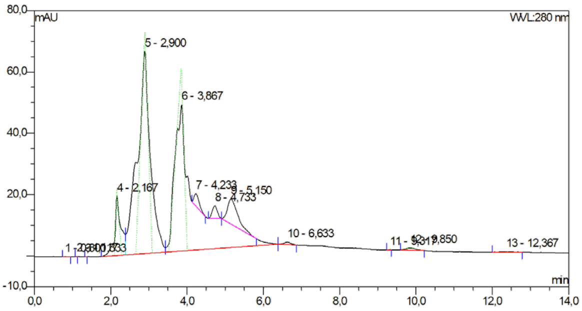

**Fig.2.** HPLC absorption diagram at 280 nm obtained from ethanolic leaves and fruit-peels extract from the *Opuntia ficus indica.* Absica shows retention time in min and ordinate arbitrary absorption units.

As can be seen in Figure 1, a number of compounds are eluted. Two major peaks indicate the key compounds of the extract from the leaves and peels of the fruit. The two main peaks appear at 2.9 min. and at 3.867 min retention time.

Figure 2 also shows two main substances to be obtained from the extract of the ripe fruits with their seeds. The retention time of those compound areslightly different namely, the two compounds are eluted at 2.867 and 3.950 min, respectively.

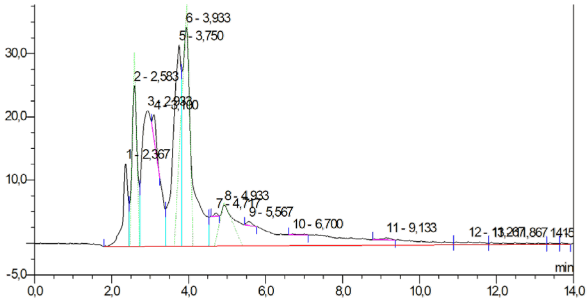

**Fig.3.** HPLC absorption diagram at 280 nm, obtained from ethanolic ripe fruits with seeds (prickly pear) extract from the *Opuntia ficus indica.* Absica shows retention time in min and ordinate arbitrary absorption units

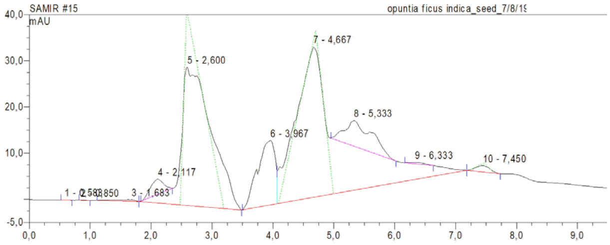

**Fig.4.** HPLC absorption diagram at 280 nm, obtained from ethanolic unripen fruit seeds extract from the *Opuntia ficus indica.* Absica shows retention time in min and ordinate arbitrary absorption units

Finally, figure 4 shows two major peaks, indicating again the existence of two main substances to be obtained from the extract of the unripen fruit seeds. The retention time of those compounds are significantly different that the other two. The main substances of the extract from the seeds give two main peaks at 2.6 min and at 4.667 min.

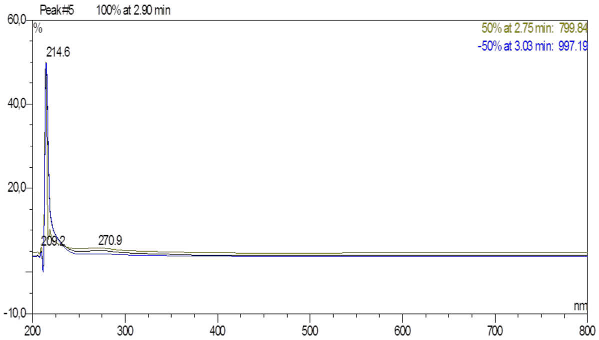

**Fig. 5**. Typical UV spectrum of the compound of the compound with retention time of 2.9 min of an extract from the leaves and the fruit peel of *Opuntia ficus indica.*

Figures 5 and 6 show the UV spectra which correspond to the two major peaks taken from a typical extract of the leaves and the the peel of the fruit. Both demonstrate three absorption peaks at 209, 215.2 and 270.2 nm indicating that they correspond to the same compound. Further chemical analyses to identify composition of those extracts are currently underway.

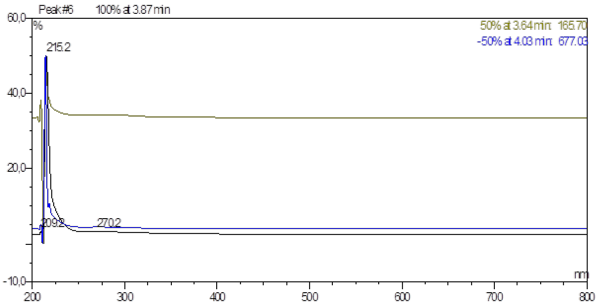

**Fig. 6**. Typical UV spectrum of the compound of the compound with retention time of 3.867 min of an extract from the leaves and the fruit peel of *Opuntia ficus indica.*

The measurement of acidity (pH) of the final extracts is shown in Table1. As can be seen, the leaf and fruit peel extracts are the most acidic of the three extracts obtained in the current study.

**Table.1.** Results from the measurement of acidity (pH)

| Extract | pH |
|---|---|
| Leaf and peel extract | 5,1 |
| Ripe fruit with seeds extract | 5,4 |
| Unripen fruit seed extract | 5,8 |

The extracts obtained via the above described methodology can be used either as individual medicinal formulations or in combination with other natural extracts to reestablish the healthy natural functions of the body.

Prickly pear is also suitable for expanding feedstock production into semi-arid marginal lands, as it represent highly water-use efficient bio-energy crop. That type of feedstock has garnered interest because of its high water- and fertilizer-use efficiency and not competing with major food crops or conventional bio-fuel feedstock [8]. Furthermore, the employed mild extraction procedure is such that key components useful for biofuel production remain unaffected. As such, the residual biomass can be further valorized via the production of chemicals, fertilizers or biofuels.

## CONCLUSION

Over the last year there has been an abundance of scientific papers on cactus pear as a source of bioactive compounds for nutrition, health and disease, underlining the interest in the numerous properties (both its bioactivity and coloring potential) of this plant species, well adapted to extreme growing conditions in arid and semi-arid zones [7].

In conclusion, the uses of prickly pear can be summarized into the following:

- ✓ Food and beverage industry
- ✓ Feed industry
- ✓ Drug industry
- ✓ Cosmetics industry
- ✓ Food supplements industry
- ✓ Manufacture of natural additives
- ✓ Energy sector
- ✓ Fertilizers

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# Phase stability analysis of multicomponent systems using alternative stochastic optimization methods

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The thermodynamic behavior of mixtures is fundamental for the process systems engineering. Phase stability analysis is a key step for process design and it implies the global minimization of Tangent Plane Distance Function (TPDF). This thermodynamic calculation is challenging and effective global optimization methods are required. In this paper, two novel stochastic global optimization techniques, namely Gray Wolf Optimization (GWO) and Water Cycle Algorithm (WCA), were tested and compared for solving phase stability problems of non-reactive mixtures. A set of benchmark problems with different thermodynamic models was considered and the numerical performance of tested optimization methods have been discussed. Hybridization of the GWO stochastic methods have been analyzed to improve its ability to find the global minimum of TPDF. Results showed that that WCA has a better performance compared to the GWO method even using the hybridized algorithm.

**Key words:** Phase stability analysis, Water Cycle Algorithm, Gray Wolf Optimization, Thermodynamic modeling.

## INTRODUCTION

Phase equilibrium behavior of pure compounds and mixtures plays an important role in process systems engineering [1]. The knowledge and analysis of the phase equilibrium is important for the design, simulation and optimization of process units such as distillation and extraction columns, reactors and absorbers. The characterization of the phase equilibrium behavior can be performed experimentally with its subsequent modeling and prediction using appropriate thermodynamic tools.

The modeling and prediction of the phase equilibrium for a mixture imply solving a fundamental problem: the phase stability analysis [2]. The problem of phase stability is solved to establish whether a multicomponent system, with a given composition at given temperature and pressure, will show phase-split [3]. The phase stability can be determined by analyzing the Gibbs free energy surface [4]. Michelsen [5] developed the Tangent Plane Distance Function (TPDF) to evaluate numerically the phase stability of mixtures. The global minimization of TPDF is used to identify the thermodynamic stability of a system. This thermodynamic calculation can be handled via the application of different mathematical tools like global optimization methods including stochastic optimizers [3,6].

Numerous global stochastic optimization methods have been reported in the literature, which can be useful for the resolution of the phase stability problem [3,7]. These methods require a very limited information about the nature of the optimization problem and can handle the discontinuity of objective functions and the presence of several local optima. The computational time of these methods is reasonable and their convergence to the global optimum is highly probable. Genetic Algorithm and Simulated Annealing, Tabu Search, Differential Evolution, Adaptive Random Search, Particle Swarm Optimization, Harmony Search and Cuckoo Search are examples of stochastic optimization methods used for this thermodynamic calculation. However, it is important to remark that stochastic optimizers still have limitations to resolve the phase equilibrium stability problem of highly non-ideal systems [3,7]. It is desirable to decrease the computational effort and to increase the effectiveness of available stochastic optimization methods that are applied in phase stability analysis [7].

The aim of this work was to test new optimization methods for resolving phase stability problems. Two new stochastic methods, Gray Wolf Optimization (GWO) [8] and Water Cycle Algorithm (WCA) [9], have been used in this study. GWO and WCA have been recently introduced to solve global a wide range of optimization problems optimization problems in engineering with promising results [8-11]. To best of the author's knowledge, these methods have not been applied in thermodynamic calculations including the modeling of phase behavior of mixtures. Therefore, these optimization

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strategies have been studied and evaluated to perform the phase stability analysis of non-reactive mixtures. Some alternatives to hybridize and improve the numerical performance of these metaheuristics are analyzed and discussed in this manuscript.

## DESCRIPTION OF OPTIMIZATION METHODS GWO AND WCA

GWO is inspired from the hierarchy leadership and hunting behavior of the grey wolves. This algorithm imitates the form of the wolves hunting that is based on the persecution, surrounding and attacking of the preys, which has been adapted to resolve global optimization problems [8]. GWO is classified as an alternative swarm intelligence optimization algorithm and, according to literature [10], it may offer several advantages such as simplicity, flexibility and derivation-free mechanism. It has also fewer control parameters to be adjusted and has a fast convergence. Some authors have concluded that GWO has better numerical properties to avoid local optima in comparison to other conventional optimization techniques and has been suggested as a suitable stochastic method for solving highly non-linear, multivariable and multimodal optimization problems [10]. On the other hand, WCA was derived by observing and emulating the water cycle process. This metaheuristic algorithm emulates the behavior of the raindrops, river and sea during the water cycle. WCA also requires few tuning parameters and it is capable of handling nonconvex objective functions with several decision variables. The advantages of WCA include its simplicity in terms of coding and implementation. Therefore, it has been applied to solve a wide range of optimization problems [11]. Results reported in different studies have shown that this novel metaheuristic is a reliable optimizer and may outperform other classical stochastic optimization methods [11]. For illustration, the flowcharts of both algorithms are reported in Figures 1 and 2.

The numerical performance of GWO and WCA was tested for the resolution of phase stability analysis using mixtures with different thermodynamic properties. Several binary and multicomponent systems were used where these problems are characterized by their non-convex and non-linear objective functions [4,7].

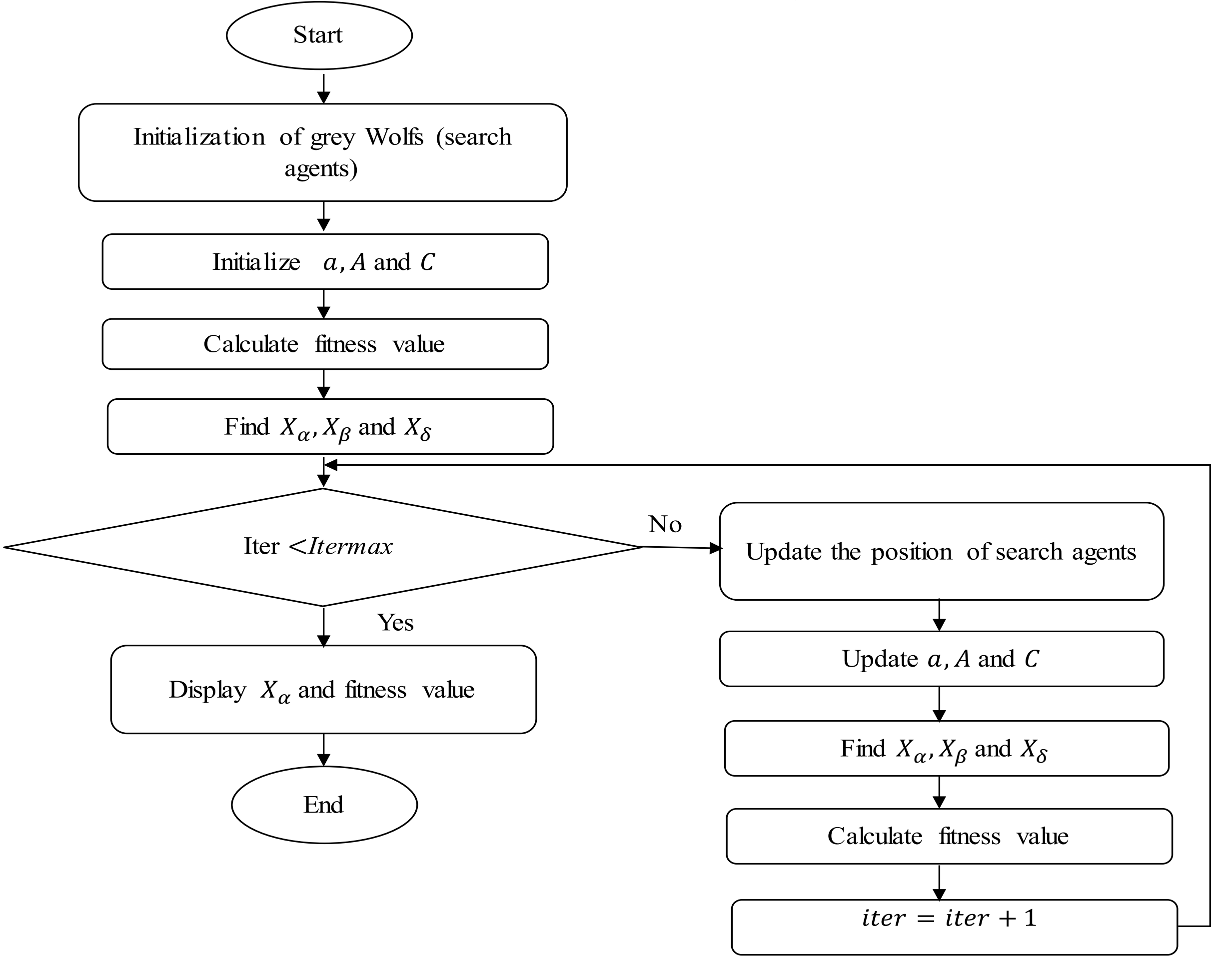

**Fig. 1.** Flowchart of Grey Wolf Optimization (GWO) method.

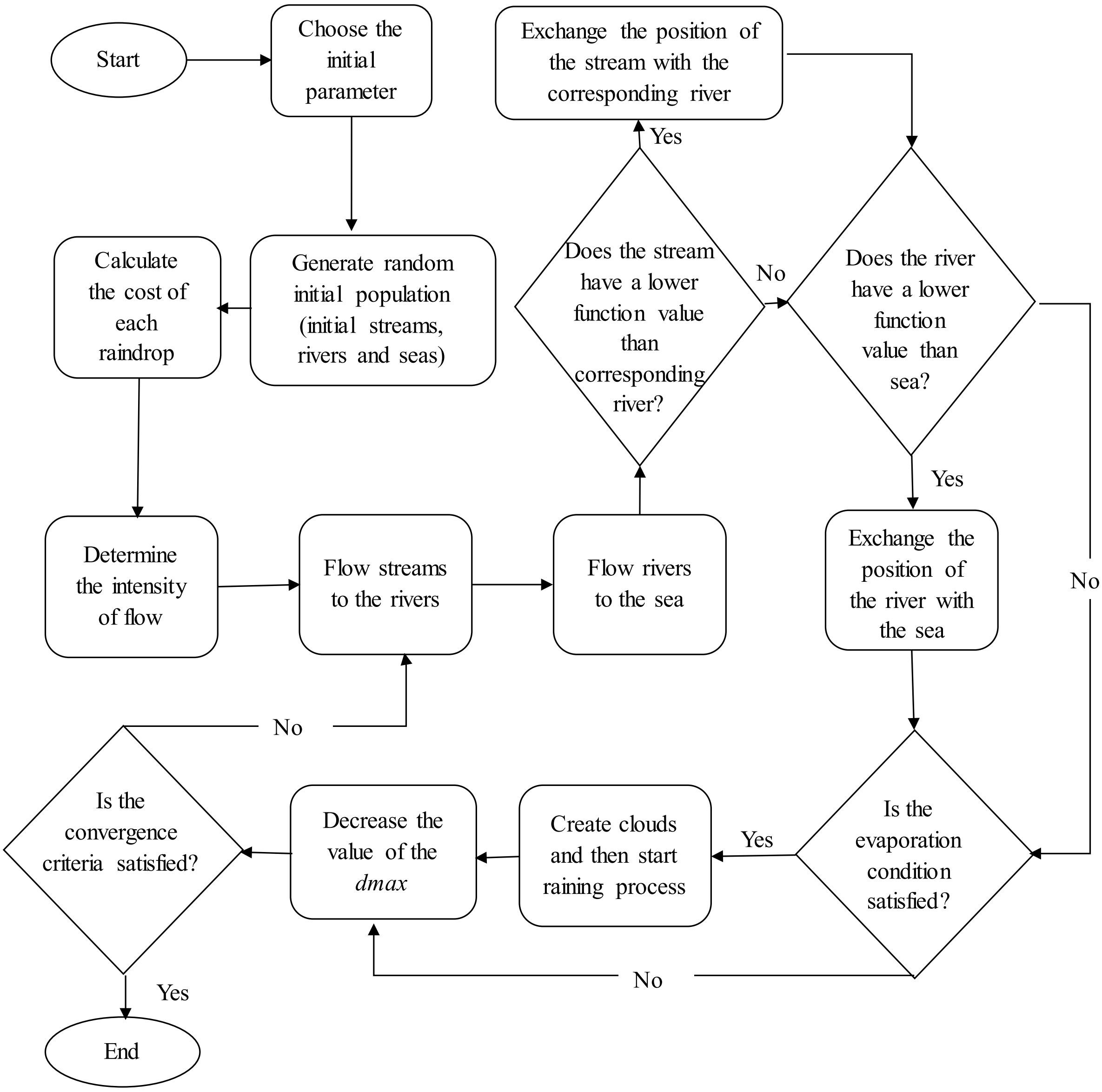

**Fig. 2.** Flowchart of Water Cycle Algorithm (WCA) method.

They include mixtures with vapor-liquid (VLE) and liquid-liquid (LLE) equilibria where the NRTL and UNIQUAC models was used for LLE and the SRK equation of state for VLE. The performance of each method in the unconstrained global minimization of TPDF has been analyzed using the success rate (SR, %), which was calculated for 100 independent trials performed for each phase stability problem with random initial values for the decision variables. Global success rate (GSR, %) was also calculated and the number of function evaluations (NFE) was employed as measure of the computational efficiency. Both stochastic methods were studied using the same stopping criterion, i.e., the maximum number of iterations *Itermax* = 100. Results of this numerical analysis were useful to identify the strengths and weaknesses of tested stochastics optimization methods in phase stability calculations.

## RESULTS

Global success rate (GSR) of GWO and WCA in tested phase stability problems is given in Figure 3. GSR ranged from 18 to 78 % for WCA and from 12 to 76 % for GWO. Results show that WCA has the best numerical behavior for solving phase stability problems independently of the thermodynamic model used in the calculation of TPDF. This method showed highest GSR than that obtained for GWO. Note that the performance of both GWO and WCA was improved after increasing NFE. It was noted that both algorithms often improved the objective function values at early NFE. In some phase stability problems, GWO and WCA provided similar results for the mean and standard deviation of the corresponding objective function. The standard deviation of both methods was relatively large in the early values of NFE. Results also showed that these stochastic methods could be trapped in local optimal values (e.g., trivial solution). For these challenging problems, WCA could improve the values of TPDF after significantly increasing NFE. It was observed that the diversification stage should be enhanced in these methods, especially, in GWO.

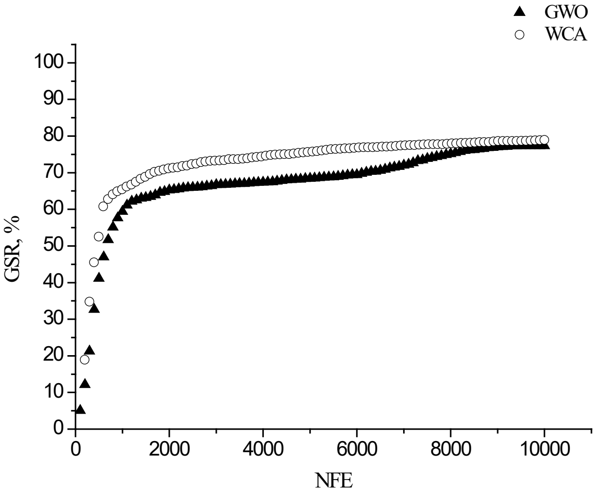

**Fig. 3.** Global success rate (GSR) of GWO and WCA for solving phase stability problems.

The performance of the GWO and WCA algorithms for locating the global minimum in phase stability problems was compared to the results reported for other metaheuristics used in the literature, see Table 1. This comparison included the next methods: Unified Bare-Bones Particle Swarm Optimization (UBBPSO), Firefly Algorithm (FA), Cuckoo Search (CS), Modified Cuckoo Search (MCS), Krill Herd algorithm (KH) and the modified Lévy-Flight Krill Herd algorithm (LKH). Results showed that the algorithm WCA was better than CS, MCS and UBBPSO and it showed a reliable performance with low NFE.

**Table 1.** Performances of WCA, GWO and other stochastic optimization methods used for phase stability analysis in nonreactive mixtures.

| *Method* | *GSR, %* | *Average NFE* |
|---|---|---|
| WCA | 88 | 25463 |
| GWO | 73 | 20512 |
| UBBPSO | 88 | 439100 |
| FA | 68 | 8461 |
| CS | 81 | 27166 |
| MCS | 76 | 41645 |
| KH | 89 | 1494 |
| LKH | 80 | 613 |

In summary, these results indicated that WCA showed a better numerical performance than GWO for solving tested thermodynamic problems. Overall, WCA could find the global minimum of TPDF with a low NFE in several non-reactive mixtures and can outperform other metaheuristics reported in other studies. Some improvements were made in GWO to strength its search mechanism. For this purpose, the operator of pitch adjustment of the Harmony Search Algorithm [12] was implemented in GWO to enhance its effectiveness in phase stability calculations. GSR of this modified GWO varied from 17 to 77 % at different numerical efforts. The combination of HS with GWO improved the diversification of original GWO allowing the exploration of more areas to find the global minimum of TPDF. Note that the convergence of this hybrid optimizer was fast in some phase stability calculations. However, this improvement was not significantly in comparison with WCA. Therefore, these results showed that WCA is the best stochastic method for the set of stability problems used in this study.

## CONCLUSION

Grey Wolf Optimization and Water Cycle Algorithm were introduced to perform thermodynamic calculations related to the phase equilibrium modeling. Results showed that WCA was capable to solve several phase stability problems with an acceptable performance. WCA required the smallest number of function evaluations to obtain a success rate similar or higher than that obtained for GWO. The performance of GWO was improved via its hybridization with Harmony Search. This new algorithm HS-GWO presented a better convergence properties for the global minimization of TPDF. However, it was outperformed by WCA, which was the best stochastic method for solving phase stability problems. This study provides insights on the application of alternative stochastic optimization methods to solve challenging thermodynamic calculations. The performance of WCA and GWO should be improved with other numerical strategies to enhance their exploration and exploitation capabilities for phase stability analysis in nonreactive systems. These improvements should be focused on their convergence properties especially to increase the reliability at a low number of function evaluations.

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# Green solvent extraction of lipids from sewage sludge of wastewater treatment plants

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This study reports the analysis of lipids extraction from sewage sludge using ethyl esters of volatile fatty acids (EEVFA). EEVFA are naturally occurring compounds, representing ones of the main constituents of flavour of fruits. They can be considered bioderivable solvents, since industrially obtainable through direct esterification of ethanol and VFAs, which can be both produced through fermentation of dedicated or exhausted feedstocks. In addition, they are easily hydrolysable in an aqueous environment, by regenerating species of origin which are completely biodegradable. For all these reasons, the use of EEVFA as extracting solvents would minimize the environmental impact respect with conventional solvents (hexane is typically used). A theoretical thermodynamic study of the lipids extraction was performed with an experimental matrix that emulated the chemical characteristics of a sewage sludge. This theoretical study was performed to obtain the phase diagrams for the lipids extraction and to identify the optimum operating conditions. A comparison of the lipids extraction using these green solvents and hexane was also performed. Then, the optimum conditions were tested and validated on real samples of sewage sludge, sampled from different municipal wastewater treatment plants. Theoretical thermodynamic studies, as well as experimental evidences confirmed that ethyl butyrate was an alternative and effective green solvent to extract lipids from sewage sludge.

**Key words:** Calcium soaps, sewage sludge, green extraction, volatile fatty acids, ethyl esters.

## INTRODUCTION

Sewage sludge is an urban waste biomass that is generated in large quantities by water treatment plants [1]. This waste has been considered as an interesting source to recovery components that can be used to obtain high added value products [2]. In particular, the sewage sludge contains a significant amount of lipids that can be converted into biofuels [3]. The lipids recovery from this biomass implies different challenges and is a fundamental stage to determine the feasibility, costs of biofuel production and environmental impact using this feedstock [4]. Lipids recovery via extraction processes is mainly affected by the physicochemical properties of the organic solvent applied [5-7]. Hexane is the most preferred organic solvent adopted for extracting lipids from biomasses, but it has a huge impact and several drawbacks [8]. Therefore, this study reports the analysis of lipids extraction from sewage sludge using ethyl esters of volatile fatty acids as green solvents. Ethyl esters of volatile fatty acids are natural occurring compounds, whose use in industry has been growing for the relevant biodegradability and bioderivability [9]. First, a theoretical thermodynamic study of the lipids extraction was performed with an experimental matrix that emulated the characteristics of a sewage sludge where calcium soaps were used as the lipid phase. This theoretical study was performed to obtain the phase diagrams for the lipids extraction and to identify the optimum operating conditions. A comparison of the lipids extraction using these green solvents and hexane was performed. In a second stage, the optimum conditions were tested and validated with a real sample of sewage sludge.

## MATERIALS AND METHODS

### *Composition of an urban primary sewage sludge*

Samples of urban primary sewage sludge (2-4 % wt of total solid) were monthly up-taken from the WWTP of Aguascalientes (México), which has a treatment capacity of 2000 $l.s^{-1}$ raw wastewater. The sludge was dewatered through filtration and/or centrifugation up to 8-10 % wt of total solids and fully characterized by adopting analytical procedures reported in literature [10]. 20-25 $\%_{TS}$, 10-15 $\%_{TS}$, 20-30 $\%_{TS}$ and 15-20$\%_{TS}$ were determined for lipids, cellulose, proteins and ashes respectively.

### *Preparation of calcium soaps from palm oil*

Calcium soaps were synthesized from palm oil (food grade) via a saponification reaction. The reaction system included 10 g of palm oil, 3 g of potassium hydroxide and 100 mL of ethanol.

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Saponification reaction was performed at 373 K for 1 h. The potassium salts of fatty acids were used in a second reaction for transforming these potassium soaps into calcium soaps. This reaction was performed with an aqueous solution of calcium chloride at room temperature and atmospheric pressure under constant stirring at 300 rpm for 3 h. Calcium soaps were stored at 277 K for 15 h and the solids were washed with deionized water and dried at 373 K for 24 h.

### *Determination of phase diagrams for the lipids extraction with green solvents*

Phase equilibrium data of the lipids extraction were recorded using an experimental matrix that emulated the composition of a sewage sludge obtained from a municipal wastewater treatment plant. This matrix was composed of 50 mL of deionized water, 1 g of cellulose, 2 g of protein (food grade, brand Isopure) and 2 g of calcium soaps. Hexane, ethyl acetate, ethyl propionate and ethyl butyrate were the solvents employed in the extraction studies of calcium soaps. Phase diagrams were obtained by varying the amount of the solvent where the extraction data were obtained at 297 K and pH of 6.4 ± 0.1. The solvent and the aqueous solution containing the calcium soaps were mixed for 1 h under constant stirring of 250 rpm. The organic and aqueous phases were separated, weighted and dried where the mass loss was quantified. Results of the material balance of drying process was used to calculate the amounts of hexane and ethyl acetate in the extractions studies, while gas chromatography was utilized to quantify ethyl propionate and ethyl butyrate. The dehydrated solids from extraction studies were grounded and used in a reaction with a solution of 2 g/L of heptadecanoate in methanol at 273 K for 2 h. The content of the methyl esters of fatty acids in the solution was quantified with gas chromatography. All the experiments were performed in duplicate and the average value was used for data analysis. Tie-lines for the extraction phase diagrams were calculated with material balances. A ternary system was considered for data analysis, which implied the mass composition (g) of the calcium soaps $J$, solvent $S$ and a pseudo-component $Ps$ formed by the aqueous solution containing the protein and cellulose. This approach allowed to visualize and analyze the thermodynamic data in a ternary diagram. Thermodynamic consistency of phase diagrams was verified using the Othmer-Tobias and Hand models. The modeling of theoretical phase diagrams for the calcium soap extraction was performed using an artificial neural network. Recovery of calcium soaps with different solvents was calculated and the optimum conditions for the extraction was identified.

### *Validation of the optimum conditions of the lipids extraction process using samples of sewage sludge*

The optimum extraction conditions identified in the thermodynamic theoretical studies were tested and validated with samples of real sewage sludge. Extraction studies were performed with ethyl butyrate and results were compared with those obtained using hexane, which was used as a reference solvent. 55 g of the dehydrated sludge were used in extraction studies with different amounts of tested solvents.

## ANALYSIS OF RESULTS

Characterization of urban sewage sludge carried out on samples up-taken from the urban WWTP of Aguascalientes (México), confirmed results already reported concerning the nature and the amount of the lipid phase [11]. In detail, lipids were always ranged among 20-25%TS and they were mainly constituted by calcium soaps per over 85%. For this reason, the optimization study of the extraction of lipids from sewage sludge has been carried out on a synthetic mixture, whose composition respected the typical profile in terms of lipids, cellulose and proteins of a primary sludge. Considering that lipid in primary sludge were principally calcium soaps with a high content of saturated fatty acids, Palm oil (food grade) was used for preparing synthetic calcium soaps. XRD and FTIR spectroscopy were utilized to characterize these calcium fatty soaps, whereas the profile of fatty acids was defined through gas-chromatographic determination (Figure 1).

FTIR analysis presents a high diagnostic power in identifying in the carbonyl region the asymmetric stretching of carbonyl which appears typically as a couple of bands at 1576 and 1540 cm$^{-1}$ as already reported [12,13]. Once that "synthetic" fatty calcium soaps were prepared, synthetic sludge was further prepared by mixing to calcium soaps the right amount of cellulose and proteins. Then, optimization of extracting procedure was carried out. All phase diagrams can be classed as Type I where a two-phase region was present. Extraction phase diagrams indicated that the two-phase region obtained with hexane and ethyl butyrate was greater than those of ethyl acetate and ethyl propionate. Differences in the extraction phase diagrams can be associated to the polarity and water solubility of tested solvents. Determination coefficients ($R^2$)

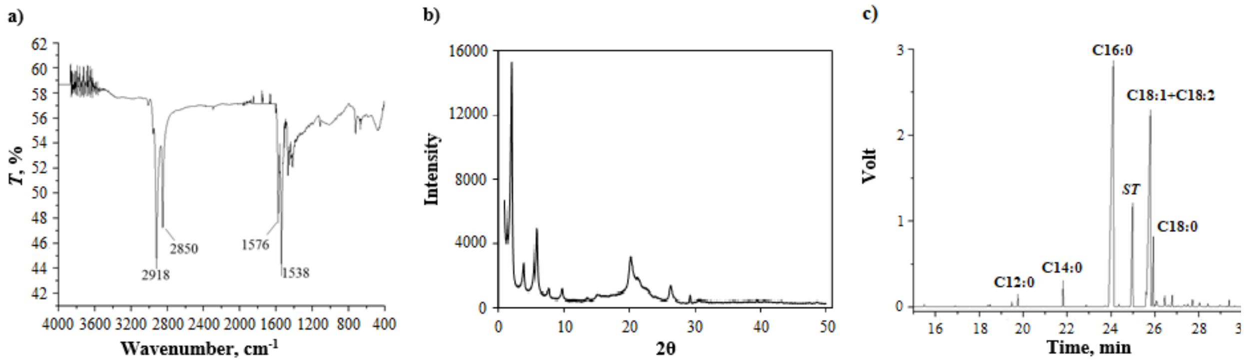

**Fig. 1.** a) FTIR spectrum, b) X-ray diffraction pattern of calcium soaps and c) GC spectra of the product obtained from the reaction of calcium soaps and methanol in presence of methyl heptadecanoate as internal standard.

higher than 0.82 were obtained for the thermodynamic consistency assessment with both Othmer-Tobias and Hand models. Recovery of calcium soaps ranged from 82 to 98 % using 4 – 44.5 g of tested solvents. Hexane and ethyl butyrate were the best solvents and showed the highest recoveries in the theoretical extraction studies of the calcium soaps. The artificial neural network model was reliable to correlate and predict these extraction phase diagrams. Finally, the performance of hexane and ethyl butyrate using samples of sewage sludge was confirmed where the lipids recoveries were higher than 90%. However, ethyl butyrate is a bioderivable solvent that can offer additional advantages, mainly in terms of environmental impacts, in comparison with hexane

## CONCLUSION

The use of ethyl esters of volatile fatty acids for the recovery of lipids from sewage sludge has been analyzed and compared. Theoretical thermodynamic studies confirmed that ethyl butyrate was an alternative and effective green solvent to extract calcium soaps. This green solvent showed high lipids recoveries in experimental matrix and samples of sewage sludge.

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# *Biorefinery and biofuels*

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# An economically viable two-step process for biodiesel production from waste cooking oils

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A real sample of waste cooking oil having an acidity of 8 $mg_{KOH}/g_{oil}$ and a water content of 700 ppm was efficiently converted into biodiesel. The process consisted in a two-step reaction: a preliminary treatment with $AlCl_3 \cdot 6H_2O$ to convert free fatty acids into the respective methyl esters, followed by alkaline transesterification using KOH as a catalyst. In detail, pretreatment with $AlCl_3 \cdot 6H_2O$ (345 K, 4 h, weight ratio MeOH:oil:catalyst of 1:3:0.01) allowed to obtain an efficient conversion of starting free fatty acid into the relevant fatty acid methyl esters (yield > 95%). After the reaction a convenient separation of phases was obtained. Two different phases were isolated: an oily phase with a methanol content of 7 % wt, in which methyl esters were dissolved in, with most of glycerides and with a very limited content of water to one side, and a lighter phase, in which most of unreacted methanol and catalyst together with water were solubilized. Such a separation made possible the direct use of the pretreated oily phase with $AlCl_3 \cdot 6H_2O$ in the transesterification without any further treatments, unless to adequate the methanol content. KOH was used to complete the conversion in the second step. Reaction occurred completely in 4 hours and the final biodiesel was conform to EN14214.

**Keywords:** Biodiesel, waste cooking oils, esterification, transesterification.

## INTRODUCTION

Biodiesel is a mixture of fatty acid methyl esters (FAMEs) which have physical and chemical properties that are very similar to petroleum-derived-fuel [1]. Industrially produced by transesterification of refined vegetal oil with methanol in presence of homogeneous alkaline catalysts, namely sodium or potassium hydroxides, carbonates or alkoxides [2], it suffers from being applied on feedstock with high free fatty acid (FFAs) (> 0.5 %wt) and moisture contents [3].

The use of refined oily feedstock generated an economic constraint for the feasibility of the entire production. 80-85% of the total biodiesel cost depended by the feedstock cost. In addition, an inappropriate change of use of land for cultivating oily plants, generated an ethical question land-for-food vs land-for-fuel. For these reason, alternative oily biomasses were investigated, for example waste cooking oils. Waste cooking oils price is 2-3 times cheaper than refined vegetable oils [4]. Besides the economic and environmental benefits, the use of WCO presents some challenges, related to the presence of free fatty acids (FFAs) and high moisture content. A high presence of FFAs (> 1 %) impedes the use of alkaline transesterification, for the concomitant production of soaps. In that case, a two-step process is normally recommended. In this two-step process, a pre-treatment (step 1) is used to reduce the amount of FFAs, through an acid catalyzed direct esterification to obtain FAMEs. Sulfuric acid is typically adopted, but its use need some intermediate processes (washing, neutralization, etc.) among pretreatment and transesterification that results in production of waste and consume of energy [5]. The esterified feedstock is converted in biodiesel through alkali catalyzed transesterification in the second step of the process. In this work, a two-step process was reported in which direct esterification of FFAs is performed under $AlCl_3 \cdot 6H_2O$ catalysis, while the second phase was run in batch through KOH.

## MATERIALS AND METHODS

### *Feedstock and reagents*

The waste cooking oil feedstock was provided by GF Energy, S.A. Kiffisias 56, P.C. 15125 Maroussi, Athens, Greece. This oil was characterized in terms of water content (700 ppm), acid value (8.02 mg KOH/g), mono- (0.8 ± 0.1 %wt), di- (3.9 ± 0.3 %wt) and tri-glycerides (90.9 ± 0.3 %wt) and ashes (100 ppm).

Chemical reagents of analytical grade were used directly without further purification or treatment.

All experiments were performed in triplicate, allowing the average value and the standard deviations to be calculated.

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### *Water content and FFA content determination*

Water content was determined according to the ISO 11465 method [6].

Free fatty acids content were determined by dissolving 2 g of sample in 50 mL of diethyl-ether:ethanol solution (1:1 v:v), and 0.1 mL of phenolphthalein indicator were placed into a 250 mL flask. Then, the resultant organic mixture was titrated with 0.1 N KOH solution until a phenolphthalein endpoint (pink coloration persisted for at least 30 s) was reached. The results were expressed as milligrams of KOH required to neutralize 1 g of oil (mg KOH/g) [7].

### *Identification of the methyl esters*

Identification of the different methyl esters were carried out by gas chromatography-mass spectroscopy (GC-MS) using a Perking Elmer Clarus 500 equipped with a Clarus spectrometer.

Quantitative determinations were performed using a Varian 3800 GC-FID: FAMEs were determined using methyl heptadecanoate as internal standard, while mono-, di- and triglycerides were determined respect to 1,2,3-tricaproyilglycerol (tricaprin) after proper derivatization with pyridine and N-methyl-N-trimethylsyliltrifluoroacetamide (MSTFA). Both instruments were configured for cold on-column injections with a HP-5MS capillary column (30 m; Ø 0.32 mm; 0.25 µm film).

### *Esterification of the WCO*

The direct esterification reaction was carried out in a 500 mL Pyrex reactor using $AlCl_3{\cdot}6H_2O$ as a catalyst. 1.00 g $AlCl_3{\cdot}6H_2O$ was dissolved into 100 g of methanol, then 300 g WCO was added in order to have a final weight ratio ($R_w$) of oil to methanol equal to 3:1. The reactor was closed and placed into a thermostatic bath at 343 K for 4 h under agitation using a magnetic stirrer. At the end of the reaction, the system was cooled and a bi-phasic system composed by an upper methanolic phase and a lower oily phase was observed. The two phases were separated, weighed, and analysed for methanol (gas-chromatographically determined), water (loss of weight at 378 K for 24 h), ash, metals, residual acidity, FAMEs, mono-, di- and tri-glycerides content. The oily phase recovered from such a pretreatment was analysed and then directly used in the transesterification.

### *Transesterification of pretreated WCO with methanol using KOH as a catalyst*

10.0 g oily phase obtained from pretreatment of WCO with $AlCl_3{\cdot}6H_2O$ was directly weighted into a 20 mL glass reactor equipped with a silicone septum, which allowed sampling throughout the reaction without interruption, agitation and heating the system. 5 g of methanol was then added.

The reactor was closed, introduced into a thermostatic oil bath (343 K) and magnetically stirred (400 rpm). Reaction time started when a previously prepared 0.84 KOH in 1.82 g MeOH solution was introduced via syringe into the reactor. Samples (0.5 mL) were up-taken after 1, 2.5, 5, 10, 15, 30, 60, 120, 240 and 480 minutes, and transferred into vials, in which 0.05 g glacial acetic acid was previously weighted to immediately quench the transesterification. Then, methanol was evaporated and residual oil was analysed.

## ANALYSIS OF RESULTS

$AlCl_3{\cdot}6H_2O$ was used as a catalyst for pretreating WCO containing 8 mgKOH/g oil.

After 4 h at 345 K, residual acidity become 0.85 mgKOH/g oil: about 90 % of initial FFAs were converted into FAMEs.

$AlCl_3{\cdot}6H_2O$ not only promoted the reaction, but induced at the end, a separation of phases with a very convenient distribution of final products. Catalyst was completely dissolved in the alcoholic phase, together with most of water, whereas the resulting methyl esters were dissolved into the oily phase. This composition made the pretreated oil a suitable feedstock to be transesterified through conventional basic catalysts without further treatments and without generating salty waste. In fact, the direct use of KOH as a catalyst was tested. Concentration of FAMEs, mono-, di- and triglycerides were monitored along the time. Results are reported in Fig. 1.

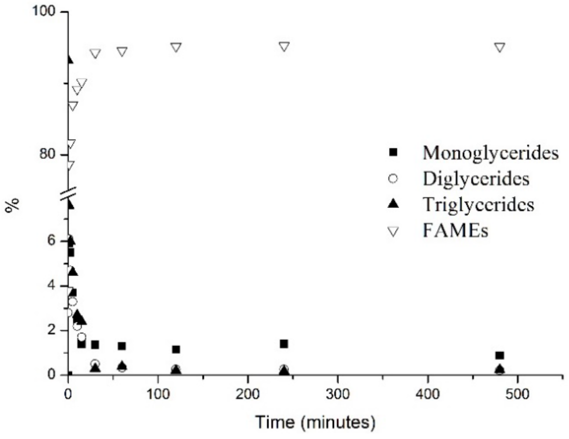

**Fig. 1:** Time course of FAMEs, mono-, di- and triglycerides

Transesterification occurred really fast: in about 30 minutes, FAMEs content was 95 % approximately and an almost complete conversion of glycerides were obtained.

The results obtained open the possibility to develop a two-step process based on two batches in series with the following benefits:

1. a sustainable route to produce biodiesel from low-cost feedstocks
2. an esterification process based on hydrated salts that allow the recovery of the catalyst and remove the washing steps used with traditional acid catalysts
3. a directly usable esterified oily phase to be treated with KOH for the transesterification step
4. a reduced number of equipment to develop the whole process
5. the possibility to decrease the capital and operative costs connected to the biodiesel production

## CONCLUSIONS

This work describes a very efficient chemical two-step process for the conversion of waste cooking oil into biodiesel. Cheap and safe $AlCl_3 \cdot 6H_2O$ efficiently promoted direct esterification of FFAs into methyl esters. The direct esterification reaction took place in the methanolic phase, in which the catalyst was completely soluble (homogeneous catalysis). At the end of the pre-treatment, it was totally recovered from the methanol phase. Besides the conversion of FFAs, a concomitant reduction of the starting water content in the reacted oily phase made the pre-treated oily phase a suitable feedstock to be directly used in the next step for obtaining biodiesel. Alkaline transesterification using KOH without any further treatment was used for the second step. At the end, biodiesel compliant with EN14214 specifications was isolated.

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# The role of biomass pretreatment for sustainable biorefineries

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Pretreatment has been regarded as one of the most critical steps for the development of sustainable biorefineries that are based on lignocellulose conversion processes and this is related to the close association that exists among plant cell wall macromolecular components. Among the many criteria for determining the efficiency of a biomass pretreatment technique, the obtainment of high process yields in fermentable sugars and other useful process streams, the development of substrate accessibility for enzymatic hydrolysis and the minimal release of both hydrolysis and fermentation inhibitors are highly influential. This work summarizes the main principles of the most relevant biomass pretreatment techniques for biorefinery applications.

**Key words:** lignocellulosic materials, pretreatment, biorefinery, process yields

## INTRODUCTION

Biomass recalcitrance is the major obstacle towards the transformation of lignocellulosic materials into valuable products. Several pretreatment techniques have been made available so far for different feedstocks and for different purposes [1-3]. However, choosing the right pretreatment technique requires a broad knowledge about the biomass chemical composition, the process steps to which it will be submitted, and the downstream processing involved in achieving products that satisfy the market demand for quality, yields and environmental impact. Parameters such as process energy balance, capital cost for operations in large scale, ability of being multipurpose in relation to feedstocks and products, cost of the biomass source and environmental implications of the downstream processing must be ideally matched to define a highly efficient, low-cost pretreatment technique for biorefinery applications [4-6]. Therefore, one needs to know the fundamental chemistry and basic engineering of a given pretreatment technique to be able to diversify its application as much as possible to different raw materials and to a variety of desired products.

## PRETREATMENT TECHNIQUES FOR OPTIMAL BIOMASS DECONSTRUCTION

The recent trend of transforming conventional industrial facilities to platforms of a wide range of bioproducts using the concept of biorefinery presents an increasing socioeconomic and environmental appeal. In addition, the possibility of developing biomass production processes following the pillars of circular economy stimulates stakeholders and government funding agencies, targeting further developments to improve the economics of the entire production chain [3, 5-8]. Therefore, several research strategies are being devoted to the development of more efficient conversion processes and to the improvement of those already existing.

The use of renewable biomass for bioproducts or bioenergy applications covers a series of steps from logistics to process optimization biomass harvesting and conditioning, pretreatment, enzymatic hydrolysis, fermentation and ethanol separation [1, 9]. By contrast to first generation ethanol production processes (mostly based on sucrose and starch hydrolysates), pretreatment is a crucial step to reduce the natural recalcitrance of lignocellulosic materials, leading to higher cellulose and hemicellulose availability for hydrolysis and consequently to higher yield monomeric sugars yields for fermentation.

Pretreatment techniques range from relatively simple operations such as drying, grinding or milling and sieving, regarded as mechanical or physical pretreatments, to more sophisticated approaches using specialty chemicals and complex unit operations. There is a wide range of mature pretreatment techniques already available and these are based on the use of diluted acids, alkali, ammonia, ionic liquids (ILs), organic solvents, liquid hot water and supercritical fluids, just to name a few [3, 6, 10-12]. Currently, many research groups are exploring this research topic and several elegant bibliographic reviews have been made available in the literature [1, 13-17].

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Beyond recalcitrance minimization for the efficient enzymatic hydrolysis of plant cell wall glucans (mostly cellulose), pretreatment can also allow for the recovery of desired co-products, such as extractives, hemicelluloses, oligomers and specially lignin. Lignin is a biopolymer that stands out as a rich matrix for the production of numerous high value-added products for energy or fine chemical applications [18-20].

Broadly speaking, pretreatment techniques are classified into physical, chemical, physicochemical, biological or hybrid processes in which two or more of these options are combined [9, 21, 22]. By contrast, some authors prefer to classify pretreatment approaches according to their effect on biomass structure, that is, size reduction, dissolution, hydrolysis, oxidation, reactive extraction or biodegradation [14, 23, 24]. Additionally, there is a classification based on pretreatment pH (alkaline, acid or neutral) [25]. Indeed, achieving a highly effective biomass fractionation by employing one single pretreatment technique is a difficult task and still a great industrial challenge. Therefore, a combined approach is highly recommended to achieve high yields of cellulose fibers, sugar monomers and lignin among other biobased products of interest [8, 26, 27]. Table 1 describes some of the most important pretreatment techniques according to their broad classification and provides details about their main effect on the macromolecular organization of lignocellulosic materials.

**Table 1.** The main pretreatment techniques applied to lignocellulosic materials.

| Process | | Description |
|---|---|---|
| BIOLOGICAL | Fungi | Lignin biodegradation by laccases and manganese peroxidases. High selectivity at very long pretreatment times |
| | Bacteria | Genetically modified organisms that are able to convert biomass into fuels and chemicals (consolidated bioprocessing) |
| | Enzymes | Selective removal of high molar mass components in cellulosic matrices using lipases, lignin-degrading enzymes and hydrolases |
| PHYSICAL | Milling | Reduction of particle size and increase in substrate surface area for biological or chemical conversion processes |
| | Microwave | Disruption and swelling facilitating hemicelluloses and lignin removal; heating and reaction times are greatly reduced |
| | Ultrasound | Structure modification by cavitation; bonds in lignin-carbohydrate complexes are cleaved by radical chemistry |
| CHEMICAL | Concentrated acid hydrolysis | Cellulose swelling and partial hemicellulose hydrolysis; lignin coalescence and condensation |
| | Dilute acid hydrolysis | Cellulose accessibility increased by partial hemicellulose removal; lignin coalescence, fragmentation and condensation |
| | Alkaline extraction | Lignin extraction and partial hydrolysis of aryl-ether bonds, reducing its average molar mass |
| | Ionic liquids | Carbohydrate or lignin extraction due to its high polarity and strong intermolecular interactions with the cellulosic matrix |
| | Oxidation | Delignification with strong oxidants such as hypochlorite, oxygen radicals and ozone |
| | Supercritical $CO_2$ | Partial acid hydrolysis of hemicelluloses, increase in substrate pore volume and available surface area |
| COMBINED | Organosolv | Selective biomass delignification, whose efficiency can be increased by adding an exogenous acid catalyst |
| | Kraft pulping | Alkaline delignification of lignocellulose at ~170 °C using aqueous $Na_2S$/NaOH to isolate cellulose fibers (holocellulose) |
| | Sulfite pulping | Acid delignification at ~160 °C using sulfite/bisulfite species to isolate almost pure cellulose fibers and lignin as lignosulfonate |
| | SPORL | Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose, developed from sulfite pulping to improve enzymatic hydrolysis |
| | AFEX | Partial removal of hemicelluloses and lignin plus changes in the crystalline state of cellulose (from I to III) |
| | Hydrothermal pretreatment<br>Liquid hot water | Hemicellulose removal and lignin fragmentation and redistribution by autohydrolysis, increasing cellulose accessibility to enzymatic hydrolysis and hemicellulose recovery mostly as water-soluble oligosaccharides |
| | Steam explosion | Acid hydrolysis of hemicelluloses and lignin modification and redistribution; may be assisted by acid or basic catalysts |

Disrupting the cell wall to facilitate enzymatic hydrolysis without sugar degradation and generation of fermentation inhibitors is the main goal of an ideal pretreatment. As described in literature [9, 28], the assessment of biomass pretreatment processes is complex and depends on several factors including (i) the total amount of recoverable carbohydrates (monomers and oligomers released in the liquid phase and polysaccharides retained in the water-insoluble solids); (ii) the accessibility of either unwashed or water-washed pretreatment solids to enzymatic hydrolysis; (iii) the fermentability of pretreatment hydrolysates (C5 stream), diluted or concentrated, targeting microbial growth and process yields; (iv) the fermentability of enzymatic hydrolysates from whole slurries or water-washed pretreatment solids; and (v) the assessment of additional chemical/biotechnological applications of the pretreated fractions for the development of high value-added coproducts [9, 28].

Pretreatment has been recognized as the most important step towards the development of sustainable biomass conversion processes such as cellulosic ethanol production. Biomass cultivation, harvesting and preconditioning, enzymatic hydrolysis, fermentation and product recovery are all very important as well, but none of them matches the strategic planning involved in choosing the best pretreatment and its ideal operational conditions for a given biomass type [8, 10]. Under the biorefinery concept, pretreatment is critical to determine how agnostic is the conversion technology regarding the biomass source and its presentation, with details such as energy balance, consumption of chemicals and generation of enzyme and fermentation inhibitors being paramount for determining its technical and economic viability [4, 8]. Therefore, by choosing the right pretreatment method and by executing it under its optimal conditions, one may meet the basic definition of advanced biorefineries, whereby multiple feedstocks can be converted to several value-added products ranging from fuels, chemicals and materials with wide applications in industry and in the transportation sector.

## MAIN PRODUCTS FROM BIOREFINING OF LIGNOCELLULOSIC MATERIALS

According to the National Renewable Energy Laboratory (NREL, USA), biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals [29]. The main driver for the establishment of biorefineries is process sustainability. Hence, this assessment must take into account the food *vs.* fuel dilemma, the land-use and water requirement for primary production and the impact on soil fertility, biodiversity and greenhouse gas emissions [7, 29].

Biorefinery has been suggested to provide relevant substitutes to a number of products derived from crude oil. In general, biorefineries can be classified in three different types. Type 1 (or first-generation) has little or no feedstock flexibility and a limited processing capability to produce fuels and co-products. Type 2 (or second-generation) has wider options in end-products but little flexibility in the incoming feedstock. Finally, type 3 (or third-generation) is flexible for both feedstocks and end-products, being able to exploit locally available biomasses to produce a variety of industrially attractive products and/or materials [30]. This advanced biorefining facility could produce one or several low-volume, high-value chemicals and a low-value, high-volume liquid transportation fuel, while generating process heat and electricity for its own use and for selling it to the grid. In this configuration, power production would reduce costs and avoid greenhouse gas emissions, high-value chemicals would enhance profitability and bulk liquid biofuels would feed the market to meet the national energy demand.

Another way of classifying biorefineries may be related to the type of incoming feedstock in a similar way to what has been applied to biofuels. High-value edible feedstocks such as neutral lipids, starch and sucrose are converted by first-generation technologies. Second-generation would apply to the utilization of non-edible lignocellulosic materials including agricultural, agroindustrial and forest residues. Finally, non-conventional feedstocks produced by fermentation processes (yeasts, fungi and microalgae) would characterize third-generation technologies for the production of fuels, chemicals and biomaterials [29, 31, 32]. However, the classification described in the previous paragraph is more consistent as it considers both feedstocks and products to classify different types of biorefinery strategies.

Pretreatment is normally required to assist biomass conversion processes that are associated to the development of type 3 biorefineries. Also, pretreatment is critical for the production of second-generation biofuels such as cellulosic ethanol [1, 6, 10]. Several process streams may be generated by pretreatment and these are tentatively summarized in Table 2. Due to the chemical composition of such feedstocks, products were organized as derived from cellulose, hemicelluloses, lignin, extractives and ashes.

**Table 2.** Main products directly obtained from pretreatment of lignocellulosics for biorefinery applications.

| Fraction | Product | Process |
|---|---|---|
| CELLULOSE | Glucose | Acid hydrolysis |
| | Dissolving pulp | Pulping and bleaching |
| | Cellulose derivatives | Pulping, bleaching and chemical modification |
| | Cellulose nanocrystals | Pulping, bleaching and enzymatic hydrolysis |
| | Cellulose nanofibers | Pulping, bleaching and mechanical refining |
| HEMICELLULOSE | Monosaccharides | Acid hydrolysis |
| | Oligosaccharides | Autohydrolysis and mild dilute acid hydrolysis |
| | Furan compounds | Carbohydrate dehydration |
| | Hydrocolloids | Partial acid hydrolysis and functionalization |
| | Liquid fuels | Fermentation after acid hydrolysis |
| LIGNIN | Phenolic acids | Hydrolysis, acid or alkaline; oxidation |
| | Vanillin | Oxidation |
| | Lignosulphonates | Sulphite pulping; SPORL |
| | Liquid fuels | Pyrolysis, followed by fraction distillation or not<br>Gasification followed by Fischer-Tropsch synthesis |
| | Solid fuels | Precipitation, drying and densification |
| | Gaseous fuels | Gasification followed by gas cleaning |
| | BTX | Pyrolysis followed by fractionation |
| EXTRACTIVES AND ASHES | Phenolic compounds<br>Organic acids<br>Food additives | Supercritical $CO_2$; solvent extraction; high pressure bleeding |
| | Fillers<br>Catalysts | Calcination |

Cellulose is a β-(1-4)-D-glucan that yields glucose by complete enzymatic hydrolysis. Acid hydrolysis can also be used for the same purpose but not without releasing 5-(hydroxymethyl)furfural by dehydration and other reaction by-products such as formic and levulinic acids as well as a range of other furan derivatives. Glucose is an easily fermentable sugar that can be converted to biofuels such as ethanol, butanol and hydrogen, as well as organic acids, ketones, higher alcohols, aminoacids, hydrocarbons and several types of biopolymers [33]. Moreover, glucose can be reduced to sorbitol and be catalytically converted to a large variety of chemicals by a wide variety of chemical routes.

Pulping processes are known as the most successful biomass conversion process available to date. Apart from pulps for paper making, other high value-added products can also be produced. For instance, dissolving pulp is a high-quality pulp product containing high cellulose (90%) and low hemicellulose (4%) contents, and trace levels of other biomass-derived components [33]. This product can be used to produce viscose rayon, nanocellulose and cellulose derivatives such as acetate, nitrate and ethers that have broad markets in the fields of textile, military industry, chemical industry, food, coatings and in material science [34]. The market demand for dissolving pulp is growing steadily in the past few years. For instance, the global production capacity of dissolving pulp was about 8.4 million tons in 2017, and China imported as much as 2.6 million tons [35].

Among the many opportunities arising from biomass conversion processes, cellulose nanomaterials are considered of high economic value and great general interest by several industrial sectors including textiles, food and pulp and paper. These products were classified by TAPPI (Technical Association of the Pulp and Paper Industry, USA), accordingly to proposed standards terms and definitions for cellulose nanomaterial WI3021 [36] in two different categories as (a) cellulose nanofibers (CNF), whose diameters are below 100 nm, and (b) cellulose nanocrystals (CNC), whose diameters and lengths are below 100 nm. In recent years, a greater demand for the development of a biobased economy has contributed to an increase in the investment for the production of nanocellulose materials that are mainly applied in paints as viscosity modifier, in coatings for better stability and homogeneous spreading), in cements as reinforcement agents [37], in automotive replaceable parts as plastic reinforcement for composites, in paper making to improve mechanical properties [38] and in packaging as barrier to control humidity and gas permeation [39].

Hemicelluloses are less-recalcitrant non-cellulosic heteropolysaccharides that are composed of several building blocks including pentoses, hexoses, uronic acids and acetyl groups [40, 41]. In this category, xylans deserve special attention because they are the most common hemicellulose found in hardwoods and herbaceous crops. As any other plant polysaccharide, xylans can be hydrolysed to monomers (mostly xylose) to produce xylitol by catalytic hydrogenation, furan compounds (mostly furfural) by carbohydrate dehydration, and liquid fuels and many other biobased products by fermentation [40]. Hemicellulose hydrolysates can also be used to produce biopolymers such as poly(lactic acid) and poly(butylene succinate) that are natural substitutes for petrochemical plastics such as polyethylene terephthalate and polybutylene terephthalate [29, 41]. Pretreatment at low severities may convert xylans into xylo-oligosaccharides (XOS) with a direct application in commercially available prebiotics. XOS can be obtained from xylans by partial acid hydrolysis, by enzymatic hydrolysis or by a combination of these [42]. For applications in the food industry, the enzymatic hydrolysis route is preferred because it does not lead to the formation of undesirable side products such as furan compounds and organic acids.

Lignin is the most recalcitrant and the second most abundant biopolymer found on Earth. This polyphenolic macromolecular component is primarily composed of phenylpropane units with varying degrees of methoxylation and its unique availability, properties and composition have been exploited in many ways to compete with and/or replace a wide range of petrochemicals [43]. Lignin can be converted to a variety of commercially valuable chemicals and materials, including hydrocarbons, cinnamic acids, phenols and catechols, benzylic aldehydes, quinones, alkyl benzenes, bio-oil, carbon fibers, activated carbon and polymeric materials such as phenol-formaldehyde resins. The processes used to break down lignin into valuable products are similar to those used in oil refineries, such as pyrolysis, acid and base (alkaline) catalytic systems, and reductive and oxidative processes [44].

A novel and promising field for lignin derivatives is in the construction of light-mass vehicles. Recently studies have demonstrated that 40 to 50% of the steel used in a vehicle could be replaced by lignin-derived carbon fibers [45]. Lignin obtained from lignocellulosic biorefineries could be an ideal precursor for carbon fibers, thereby replacing costly polymeric materials such as poly-acrylonitrile and opening a myriad of other applications in the development of biobased sustainable materials [46]. Despite its tremendous potential for biorefining, about 60% of the lignin produced nowadays primarily by *kraft* pulping is utilized for heat and power to meet the internal energy demand and to facilitate the recovery of chemicals in large scale industrial facilities [47]. However, lignin fractionation and/or depolymerization may lead to the production of several highly functionalized molecules including phenolic aldehydes such as vanillin, syringaldehyde and *p*-hydroxybenzaldehyde, phenolic ketones such as acetovanillone and acetosyringone, phenolic acids such as vanillic and syringic acids and a wide range of other chemicals [48, 49]. For a deeper knowledge about lignin utilization for fuels, chemicals and materials, please refer to some of the most recent reviews in this topic [20, 49-52].

## CONCLUSION

The recalcitrance of the plant cell wall macromolecular structure is the ultimate barrier for the development of sustainable biorefineries based on biomass conversion processes. For this reason, pretreatment and fractionation plays an important role in such process development. The first challenge is to select the right pretreatment technology for potentially available biomass sources and then to optimize it in such a way to achieve the best possible yield of a variety of marketable biobased products such as fuels, chemicals and materials.

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# Scale-up studies for intensified production of biodiesel from used cooking oil

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In this paper the effect of channel size on the transesterification of used cooking oil (UCO) with methanol using KOH as catalyst to produce biodiesel was investigated for capillaries with internal diameter ranging from 1 to 3 mm. A T-junction was used as the mixing zone of the two liquid phases. The effects of different parameters such as, internal diameter, methanol-to-oil molar ratio, reaction time, temperature, and catalyst concentration were investigated. Results showed that the conversion efficiency to biodiesel is increased by decreasing the channel size, whilst the interactions of the other variables are also discussed.

**Keywords:** Process Intensification, Scale-up, Waste cooking oil, Biodiesel, Liquid-liquid

## INTRODUCTION

Biofuels are considered as promising alternatives to fossil fuels, because of the need to reduce emitted $CO_2$, and the problems associated with the security of supply and geopolitical issues. The last decade their annual growth rate was at 10% and is expected to grow even more, while other renewable alternatives are still under development [1]. Biodiesel is one of the biofuel products that has received considerable attention and is already used in the transportation sector since it is compatible to current engine designs and performs very well in most conditions [2]. Biodiesel is a fatty acid methyl ester, mainly produced from vegetable oils of palm, soybean, coconut, and rapeseed. During the esterification process, the vegetable oil reacts with an alcohol, mostly methanol or ethanol, in the presence of a catalyst at 60 ºC [3]. Biodiesel biodegrades rapidly and is 100% non – toxic, which means that any spillages will be of far less risk compared to spillages of fossil diesel. Despite the many benefits associated with biodiesel, research is needed on its production, for a clean, effective, and environment-friendly technology to make it cost effective and increase its competency against conventional fossil fuels [4]. Another major concern is the use of edible oils for the majority of the biodiesel worldwide from crops that compete with arable land. To overcome this problem, an alternative, less expensive feedstock can be used. Options of feedstocks that are cheaper include non–edible oils and used cooking oils. The main issue with low cost oils is that they hold high levels of unwanted components including water and free fatty acids, which means that pre-processing steps should be considered in the production process. The production of biodiesel is usually carried out in liquid-liquid contactors where currently used technologies are old. Substantial process intensification can be achieved with the use of small-scale contactors where the reduced length scales result in thin fluidic films which enhance mass transfer rates, while increased surface to volume ratios improve the control of the flow patterns [5]. To increase throughput in the small scale contactors, large channel sizes should be considered that still however, preserve the benefits of small scale operations. There is a trade-off, therefore, between mass transfer performance and throughput and energy requirements, which need to be carefully considered when designing the whole process. Many works have been conducted on biodiesel production in micro-channels, the majority of which have investigated the conversion of vegetable oils. A recent review summarizes most of these works [6]. Regarding the use of waste cooking oil in the production of biodiesel, very few investigations have been conducted in the last decade. Mohadesi *et al.* [7] designed a bundle of 50 micro-reactors of 0.8 mm ID, and obtained conversion efficiency ~99% at 2 min, for methanol-to-oil ratio of 9.4:1 using KOH as catalyst. In another work, Tanawannapong *et al.* [8] investigated the production of biodiesel from waste cooking oil in a two-step reaction and found that the methyl ester content of the biodiesel was ~91%.

In this work, a one-step reaction is proposed for the transesterification of used cooking oil using KOH as a catalyst. The studies are carried out in channel sizes from 1 to 3 mm and the interaction of different variables, such as methanol-to-oil molar ratio, reaction time, temperature, and catalyst concentration are investigated.

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## MATERIALS AND METHODS

### *Materials*

Used cooking oil (UCO), with low acid value (0.02 mg KOH/g) and saponification value of 184 mg KOH/g was used. High-purity methanol, potassium hydroxide, heptane and internal standard methyl heptadecanoate were obtained from Sigma-Aldrich.

### *Experimental setup*

A schematic of the experimental setup used for the continuous production of biodiesel is shown in Fig. 1. The experiments were carried out at atmospheric pressure. Two high-precision pumps were used to feed the two phases into the main reaction channel via a T-junction mixer. The mixer was made of FEP with all branches having the same internal diameter as that of the main reaction channel. Both the reaction channel and the mixing zone were immersed in a water bath with temperature control to maintain the desired reaction temperature. At the end of the reaction channel, the two phases were collected in an iced bath to terminate the reaction and separated using a gravity funnel. The post-processing of the samples included washing (5 times with warm water) to remove any remaining residuals, and drying to remove the remaining water. The flow was visualised with a high-speed camera (Photron APX) with a maximum resolution of 1024 × 1024 at 2000 fps.

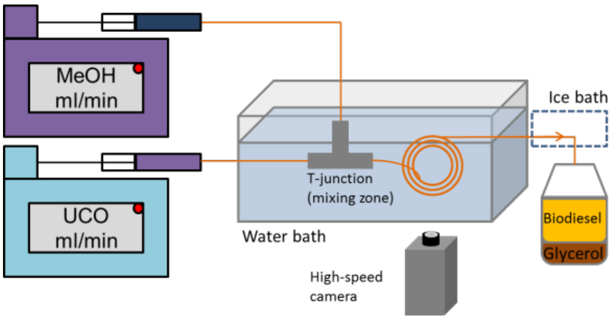

**Fig. 1.** Schematic of the experimental setup for biodiesel production

### *Analytical method.*

The free fatty methyl ester (FAME) content of the biodiesel samples was measured with gas chromatography (Agilent 6890 Series GC), using a fused silica capillary column (Stabilwax 30 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The analysis was performed following standard procedures (EN 14103) and methyl heptadecanoate was used as the internal standard.

### *Experimental design*

The effect of the different variables, i.e. internal channel diameter, oil-to-methanol molar ratio, temperature, catalyst concentration, and reaction time in the conversion efficiency of the biodiesel was investigated. The table with the minimum and maximum values of the different parameters is shown in Table 1. The total length of the reaction channels was kept constant at 1.3 m, and the flow rates of the two phases were adjusted to give the appropriate reaction time and molar ratios.

**Table 1.** Experimental conditions for the production of biodiesel.

| Variables | Range |
|---|---|
| Methanol-to-oil molar ratio | 6-18 |
| Catalyst concentration (% w/w) | 1-3 |
| Temperature (°C) | 50-70 |
| Reaction time (min) | 1-8 |
| Internal diameter | 1-3 |

## RESULTS AND DISCUSSIONS

The molar ratio is one of the most significant variables in the production of biodiesel. The transesterification reaction is reversible and thus an excess of methanol is required to move the reaction forward. Theoretically, 3 mol of methanol is required for the transesterification of 1 mol of oil to yield 3 mol of methyl ester and 1 mol of glycerol. The molar ratio, however, also affects the two-phase flow pattern in small channels. Using a T-junction to introduce the two liquid phases in the test section (Fig. 1) plug flow is established as shown in Fig. 2. When the molar ratio increased from 6 to 18 more segments were obtained, and higher interfacial area was available for mass transfer.

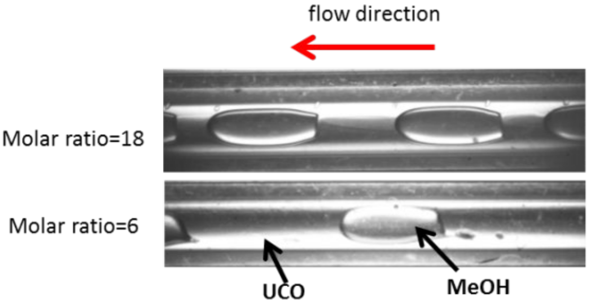

**Fig. 2.** Flow patterns in the 1 mm channel at two different molar ratios at 60 °C

Hydrodynamic and mass transfer performances are highly interdependent, so changes in the flow pattern are expected to affect the biodiesel conversion as well. In Fig. 3 the effect of molar ratio for two different channel sizes is shown. In the smaller channel (1 mm) the temperature was 60 °C, while in the bigger one it was at 70 °C. For both cases the conversion efficiency is higher at the higher molar ratio i.e. 18, even though both molar ratios are larger than the stoichiometric one. Conversion efficiency in general is higher in the smaller channel, although the temperature is not the same to allow direct comparison.

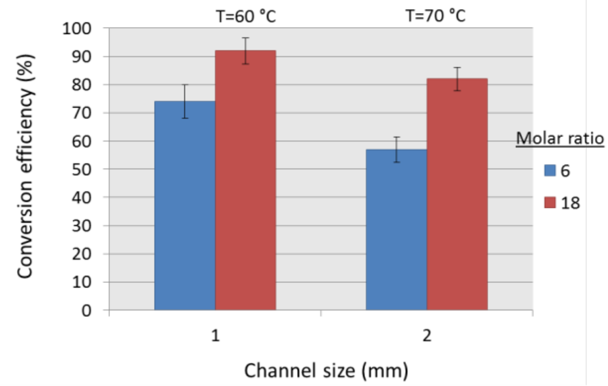

**Fig. 3.** Conversion efficiency as function of molar ratio for channel sizes 1 and 2 mm (Reaction time=4.5 min; KOH=2% w/w).

The effect of temperature is shown is Fig. 4. The transesterification reaction is an endothermic reaction, which means that the reaction rate increases with increasing temperature. However, at 50 ºC the conversion efficiency reaches >95%, while it drops to 80% at 70 ºC. This phenomenon is attributed to the fact that an increase in the temperature can also accelerate the saponification of the triglycerides (oil) by the alkaline catalyst before the completion of the alcoholysis.

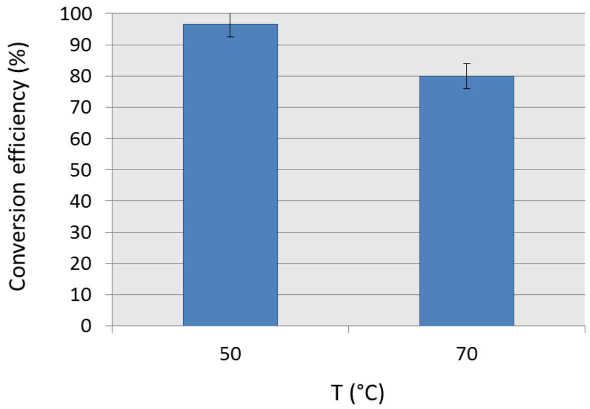

**Fig. 4.** Conversion efficiency as a function of temperature for the 1 mm channel (Molar ratio=12; Reaction time=4.5 min; KOH=2 %w/w)

The effect of catalyst concentration is shown in Fig. 5. It can be seen that for both channel sizes and temperatures, the conversion efficiency decreases by increasing the catalyst concentration.

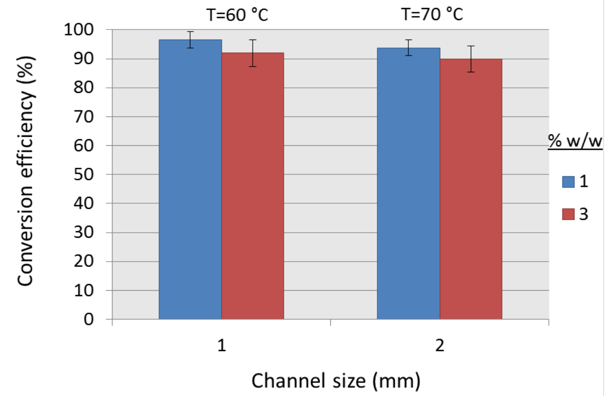

**Fig. 5.** Conversion efficiency as a function of the catalyst concentration for two channel sizes i.e.1 and 2 mm (Reaction time=4.5 min; Molar ratio=12).

However, the difference in biodiesel conversion efficiency between concentrations of 1 and 3 % w/w is very small. Increased catalyst concentration can enhance the saponification of biodiesel, but it can also affect the properties of the glycerol formed.

The reaction time also plays a crucial role in the conversion of used oil to biodiesel. By increasing the reaction time, with all the other variables constant, the conversion efficiency increases by 15% in the 2 mm channel (Fig. 6).

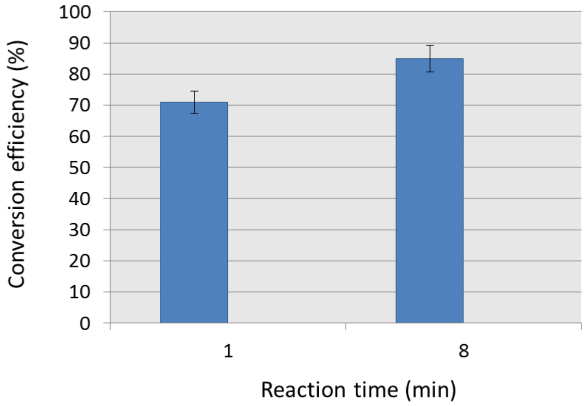

**Fig. 6.** Conversion efficiency as a function of reaction time for the 2 mm channel (Molar ratio=12; T=70 °C; KOH=2 %w/w)

To allow high throughputs, a larger channel size of 3 mm internal diameter was investigated. The conversion efficiency was compared to that of the 1 mm ID channel for a reaction time of 4.5 min, keeping all the other conditions constant. As it can be seen in Fig. 7, the conversion efficiency drops by 25% when the channel diameter increases from 1 to 3 mm.

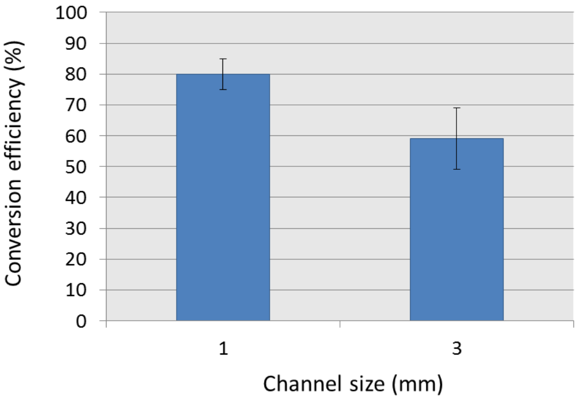

**Fig. 7.** Conversion efficiency as a function of channel size (Molar ratio=12; Reaction time=4.5 min; KOH=2 %w/w, Temperature=70 °C)

## CONCLUSIONS

In this work the effect of channel size on the production of biodiesel was investigated under different operating parameters. A one-step transesterification reaction of used cooking oil with MeOH/KOH to produce biodiesel was studied for channel sizes ranging between 1 and 3 mm internal diameter. It was found that the biodiesel conversion efficiency increased with decreasing channel size. The molar ratio affected the conversion significantly since it has a direct impact on the flow patterns that form in the small channels. An increase in the molar ratio led to an increase in the biodiesel conversion efficiency. Longer reaction times also increased the conversion.

***Acknowledgments:*** *The project was supported by the IProPBio an EU Horizon 2020 Research and Innovation Programme under the Marie Sklodowska-Curie grant agreement 778168, by an EPSRC grant on Feasibility Studies in Energy (EP/P034101/1) and by an EPSRC Impact Acceleration Account (EP/K503745)*

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# Recovery of bioalcohols with potential as biofuels using an energetically sustainable separation strategy

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The recovery of ethanol and butanol in aqueous solutions using adsorption on bone char was analyzed. Adsorption kinetics and isotherms were quantified where the effect of initial concentration, pH and temperature on alcohol adsorption was evaluated. Results showed that butanol was preferably adsorbed than ethanol due to its higher hydrophobicity. In particular, the maximum adsorption capacities of bone char to ethanol and butanol adsorption were 7.58 and 8.78 mmol/g, respectively, at pH 6 and 30 °C. It was also observed that pH and temperature significantly affected the recovery of these potential biofuels.

**Keywords:** *adsorption, ethanol, butanol, bone char, bioalcohol recovery.*

## INTRODUCTION

Biofuels are classified as a green energy source. Liquid biofuels have been proposed to form a future leading supplier of energy to replace fossil fuels like gasoline, diesel and petrol [1]. These liquid biofuels cover a wide spectrum of options including several routes for their production [2]. In particular, bioalcohols can be applied as multipurpose chemical feedstocks including their use as an energy source [3]. Ethanol is an important renewable liquid fuel for motor vehicles [4-6]. On the other hand, butanol is a chemical feedstock that has also been considered as a promising liquid fuel [6-9].

These two alcohols can be obtained by fermentation processes. However, the fermentation employed to obtain ethanol and butanol can suffer some technical drawbacks such as a high substrate cost, low product yield and high recovery costs [10]. The low product concentration in the fermentation is associated with the presence of the alcohols themselves, which are toxic to the microorganisms that carry out the process. The content of butanol and ethanol in fermentation broths usually achieves a low concentration around 2 % along with other by-products [8]. Consequently, the recovery costs of bioalcohols are highly and strongly dependent on the required product purity [11]. This aspect could be a driving factor that defines the success and the potential commercialization of bioalcohols obtained from fermentation in a biorefinery context.

It is worth mentioning that distillation is the conventional strategy to separate the alcohols from fermentation broths [12]. However, this purification method is energy-intensive; besides, the alcohol separation from aqueous systems is challenging because the presence of azeotropes increases the difficulty of the separation by simple distillation. It has been estimated that a distillation process can consume 50 – 80 % of the total energy required for the alcohol production [13]. Alternative technologies to separate alcohols from fermentation broths include the adsorption, which appears particularly attractive since it requires low energy consumption, is environmental friendly, easy to perform and the adsorbents can be regenerated and reused. Adsorption constitutes an economical method of alcohol recovery from low-in-alcohol-concentration solutions, which could contribute to reduce the production costs of this type of liquid biofuels.

This study reports the adsorption of ethanol and butanol from aqueous solutions using commercial bone char as an adsorbent, and is the first step in the development of an effective and low-cost strategy for the recovery of these liquid biofuels. The efficiency of this bioalcohol recovery strategy was tested at different operating conditions detailed in the next section.

## EXPERIMENTAL

Experiments of ethanol and butanol recovery were performed using a commercial bone char supplied by the Brazilian company Bonechar Carvao ativado do Brasil Ltda. The adsorbent was produced from bovine bones via pyrolysis, and was washed with deionized water until obtaining a constant pH

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in the washing solution. Then, it was dried and sieved to obtain a mean particle diameter of 0.35 mm (i.e., 40 - 50 mesh fraction) and this raw adsorbent was employed for alcohol adsorption.

Adsorption kinetics and isotherms for the bioalcohols recovery were experimentally quantified at different operating conditions using agitated tanks with an adsorbent dosage of 5 g/L under constant stirring. Adsorption experiments were performed at pH 6 - 7 and 20 – 30 °C using aqueous solutions prepared with anhydrous ethanol and butanol (chemical of reagent grade and supplied by J.T.Baker with CAS 64175 and 71363, respectively) and deionized water. Ethanol adsorption kinetics was performed with initial concentrations of 130 and 650 mmol/L, while initial concentrations of 134 and 670 mmol/L were used for quantifying butanol adsorption kinetics. Samples were taken at operating times from 0.25 to 24 h. Alcohol adsorption isotherms were obtained using initial concentrations from 23 to 2200 mmol/L for ethanol and from 14 to 800 mmol/L for butanol, respectively. The equilibrium time was 24 h for both alcohols. The concentrations of these alcohols were determined via gas chromatography using a Thermo Scientific Trace 1300 GC equipped with a flame ionization detector (FID). The method for alcohols quantification was adapted from procedures reported in the literature [14,15]. A linear calibration curve was utilized for the quantification of each alcohol. All the adsorption experiments were conducted in triplicate and the average values were used for data analysis. The alcohol adsorption capacity of bone char ($q$) was calculated using a mass balance

$$q = \frac{C_0 - C_t}{m} V \tag{1}$$

where $C_0$ and $C_t$ are the initial and final alcohol concentrations of the adsorption experiments, $V$ is the alcohol solution volume and $m$ is the adsorbent mass, respectively.

## RESULTS

Ethanol and butanol adsorption kinetics on bone char at different operating conditions (i.e., initial concentration and pH) are shown in Figure 1. Overall, more than 80 % of the amount of both alcohols was recovered during the first 6 h of the experiments at the operating conditions tested. These kinetic profiles indicated that the alcohols adsorption occurred on the external surface of bone char. Results given in Figures 1b and 1d showed that the higher the alcohol concentration in the aqueous solution, the faster the mass transfer and the higher the adsorption capacity. Figures 1a and 1c show that the adsorption of ethanol and butanol was significantly affected by solution pH, with the lower pH increasing substantially the adsorption capacity for either of the two alcohols. In particular, the solution pH affected the alcohol recovery because the surface charge of the adsorbent changed [16].

Figure 2 displays the adsorption isotherms of ethanol and butanol on bone char at two different pH values and two different temperatures. These isotherms were L-type according to the Giles classification, which corresponded to a favorable adsorption process [17]. The maximum adsorption capacity for ethanol was 6.36 mmol/g at 30 °C and pH 7, which increased up to 19.18 % at pH 7 and 20 °C and 19.65 % at pH 6 and 30 °C. The maximum adsorption capacity for butanol was 7.40 mmol/g at 30 °C and pH 7 and it increased 12.86 % at pH 7 and 20 °C and 16.37 % at pH 6 and 30 °C. Results showed that the adsorption capacities of butanol were higher than those obtained for ethanol. This adsorption performance can be the result of the more hydrophobic nature of butanol as opposed to that of ethanol. It is worth mentioning that longer chain alcohols show a greater adsorption capacity due to their increasingly hydrophobic nature [16]. Similar trends for the adsorption of ethanol and butanol have been reported in previous studies when polymeric resins, zeolites and activated carbon F-400 were used as adsorbents [9,18,19].

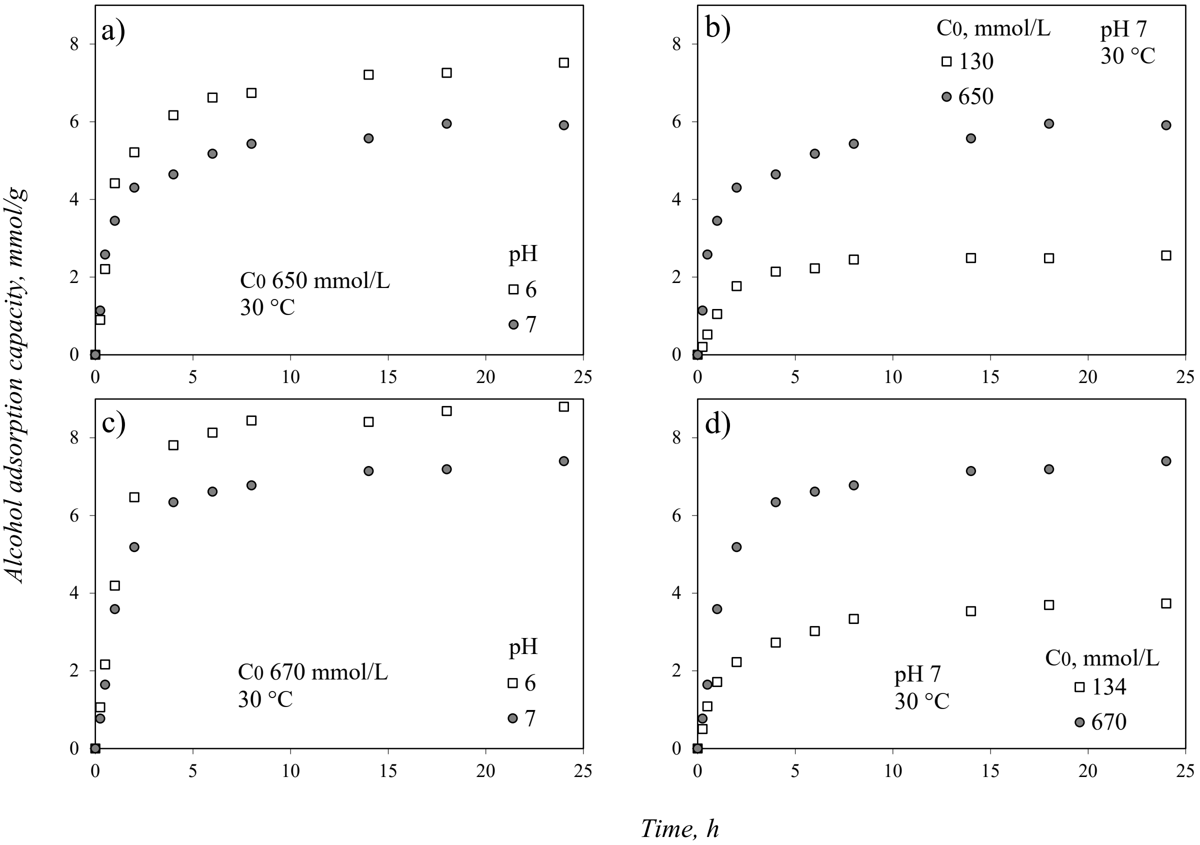

**Fig. 1.** Adsorption kinetics of a, b) ethanol and c, d) butanol at 30 °C.

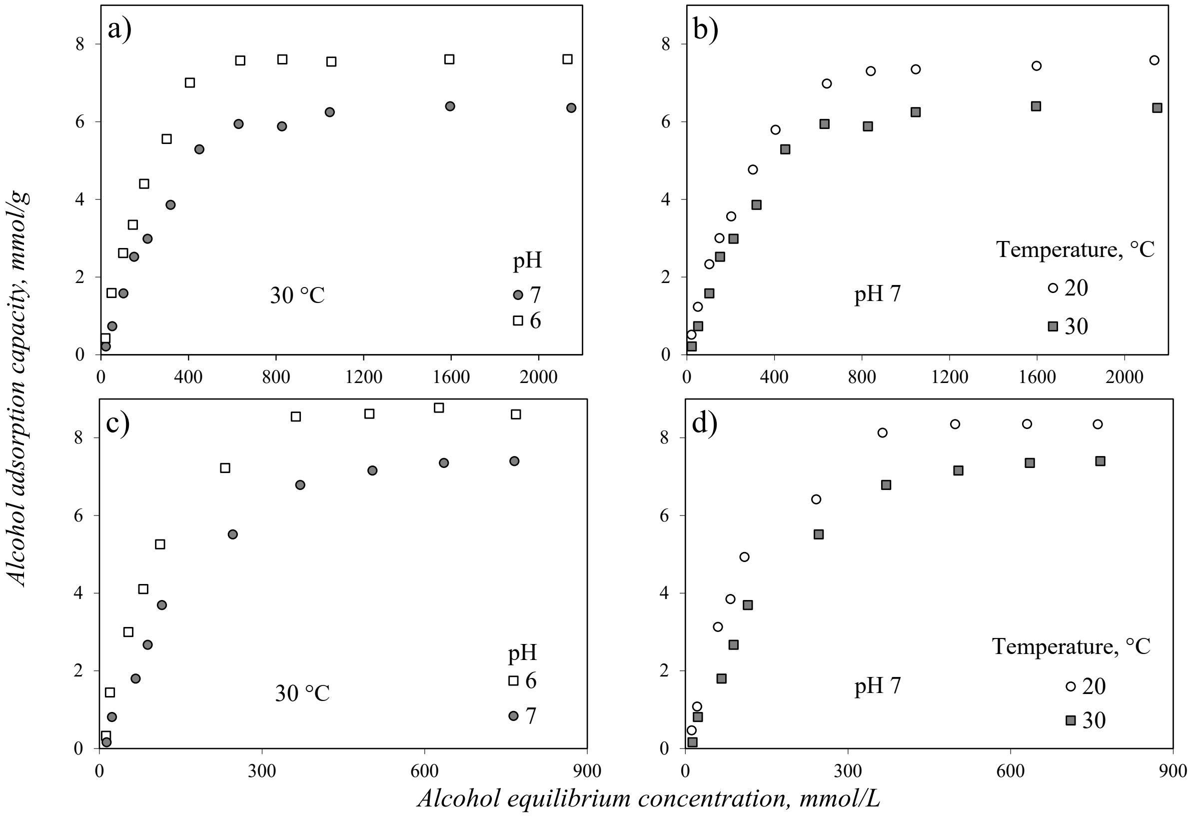

**Fig. 2.** Adsorption isotherms of a, b) ethanol and c, d) butanol at different conditions of pH and temperature.

## CONCLUSIONS

In this study, the analysis of ethanol and butanol adsorption on bone char was performed at 20 and 30 ºC and at pH 6 and 7 in a batch system. Results showed that ethanol and butanol adsorption on bone char was significantly affected by solution pH and temperature. The maximum uptake of ethanol and butanol was obtained at pH 6 and 30 °C where butanol adsorption was higher than that of ethanol. Recovery of ethanol and butanol via adsorption on bone char appears to be a feasible and low-cost process.

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# Synthesis, characterization and applications of carbon-based calcium catalysts deriving from avocado seeds for biodiesel production from waste cooking oil

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In this work, avocado seeds were successfully used as precursors to produce carbon-based calcium catalysts for biodiesel production. The catalysts were synthetized *via* precipitation method, by loading different amounts of calcium nitrate on carbonized supports obtained by pyrolysis under nitrogen flow of dried biomass. The effect of Ca loaded on the structure and the activity of the catalysts for biodiesel synthesis from sunflower oil with methanol was investigated. Results showed that supported catalyst loaded with 20%wt of Ca, efficiently promote the trans-esterification process (FAMEs content >80%) with the catalyst that was easily recovered and reused. Reaction conditions were then optimised using the desirability function applied on the response surface methodology analysis of a Box–Behnken factorial design of experiments. Finally, the optimized conditions were adopted on several non-edible oils with Free Fatty Acids content range between 1 and 15 mg KOH/g. In all cases, a FAMEs content >95% was in any case obtained.

**Key words:** Biodiesel, calcium oxide, heterogenous catalysts, avocado seeds, FFAs

## INTRODUCTION

The depletion of petroleum reserves and the growing global energy demand has led to the use of alternative renewable energy respect to petroleum-based fuels [1,2]. In Europe, several countries proposed a ban on the sale of petrol and diesel cars by making the switch to electric-powered vehicles, with many nations that will follow their lead. Biodiesel is a clean renewable fuel, which has chemical and physical properties similar to the fossil fuels [3,4]. In addition, it is biodegradable, oxygenated and free of sulfur with consequently reduced gas emissions in atmosphere compared to petroleum diesel [5,6].

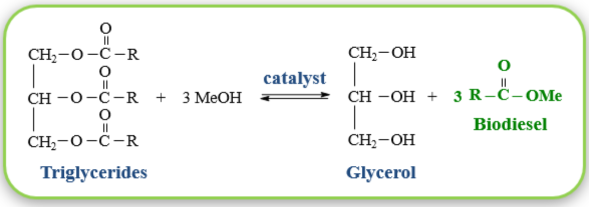

**Fig. 1.** General equation for the synthesis of Biodiesel from Triglycerides.

Biodiesel is a mixture of Fatty Acid Methyl Esters (FAMEs), typically produced by trans-esterification of glycerides contained into natural resources such as vegetable oil or animal fats with biomethanol produced from natural resources ($CO_2$, biomass, and waste [7,8]) (Figure 1), in presence of homogeneous basic catalysts (sodium or potassium hydroxide, carbonates or alkoxides [9,10]).

Such catalysts are quite sensitive to the presence of water and Free Fatty Acids (FFAs) which can be contained in small quantities in vegetable oils and animal fats. In particular, the presence of FFAs not only leads to the formation of soaps with difficulty in the separation of biodiesel produced, but also to the production of large amounts of chemical wastes [11,12]. For these reasons, refined oils or highly pure fats must be used with a consequent increase in production costs. On the other hand, homogeneous acid catalysts (hydrochloric, sulphuric and nitric acid) are no-sensible to FFAs and therefore usable for low quality feedstocks such as waste cooking oils, non-edible oils and raw animal fats [13,14]. However, they are slower respect to the basic catalysts and require reactors resistant to the acid corrosion. Thus, research efforts have been switched in the development of heterogenous catalysts [15,16]. These catalysts can be easily recovered and the end of the process and re-used for several cycles of reaction without significantly loss of catalytic activity. Hydrotalcites [17,18], mixed oxides [19,20], heteropolyacids [21], ion exchange resins [22,23] and zeolites [24] were used for the conversion of several feedstocks but for the complex expensive synthesis procedures and the reagents used, they not have still reached the full growth. Consequently, research was focused on the

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production of new "green catalysts", which can be prepared directly from biomass or its derivatives. Ligno-cellulosic biomass [25], animal bones [26] and waste shells [27] were positively used as catalysts for the biodiesel production, by significantly reducing the production costs. In addition, they are biodegradable and re-usable for several cycles of reaction. In this work, carbon-based calcium catalysts deriving from avocado seeds were synthetized and tested in the trans-esterification reaction of sunflower oil with methanol.

Once identified the most active catalyst, Box–Behnken response surface methodology was then applied to maximize FAMEs yield. Finally, the optimized conditions were adopted on several non-edible oils characterized with high FFAs content to verify the applicability of the process.

## MATERIALS AND METHODS

### *Reagents and Instruments*

All chemical reagents used in this work were of analytical grade and were used directly without further purification or treatment.

Avocado seeds (Persea Americana) were purchased from a local market of Aguascalientes (Mexico).

Carbolite Eurotherm tubular furnace was used for the synthesis of carbon-based calcium catalysts.

Identification of the different methyl esters were carried out by gas chromatography-mass spectroscopy (GC-MS) using a Perking Elmer Clarus 500 equipped with a Clarus spectrometer. Quantitative determinations were performed using a Varian 3800 GC-FID. Both instruments were configured for cold on-column injections with a HP-5MS capillary column (30 m; Ø 0.32 mm; 0.25 µm film).

### *Synthesis of carbon-based calcium catalysts*

Avocado seeds obtained from the fruit of *Persea Americana* were used as precursors for the synthesis of carbon-based calcium catalysts. Supported material was obtained by pyrolysis of the starting biomass for 2 h at 900 °C under $N_2$ flow. Carbon-based calcium catalysts were synthetized *via* precipitation method. 10 g of organic support and 11.8 g of calcium nitrate tetrahydrate (weight ratio Ca to support = 20%) were suspended into 100 mL of deionized water. Then, a sodium hydroxide solution 1.5 N was added until to obtain the precipitation of calcium hydroxide. The system was stirred for 1 h at 70 °C. Finally, the precipitate was filtered, washed with deionized water and activated for 2 h at 900 °C under $N_2$ flow. Using the same procedure, supported catalysts with 10 and 5%wt of calcium loaded were also synthetized.

### *Trans-esterification reaction of sunflower oil with methanol*

In a glass reactor of 15 mL, 2 g of sunflower oil (residual acidity = 0.21 mg KOH/g) were placed with 1.08 g of methanol (molar ratio methanol to oil = 15) and 0.1 g of catalyst (weight ratio catalyst to oil = 5%). The reaction was carried out for 3 h at 100 °C. Then, the system was cooled and the catalyst was recovered by centrifugation. The organic phase was recovered by evaporation of methanol under $N_2$ flow with the glycerol that decanted on the bottom as separate phase. Upper organic phase was recovered, washed with deionized water and dried under vacuum. Finally, methyl-esters were determined by gas-chromatography using methyl heptadecanoate as internal standard. Optimization of reaction conditions were performed using a three-level and three-factorial Box–Behnken experimental design. Amount of catalyst (2.5, 5 and 7.5%wt, $X_1$), molar ratio methanol to oil (10, 15 and 20, $X_2$), reaction time (1, 3 and 5 h, $X_3$) and temperature (60, 80 and 100 °C, $X_4$) were selected as independent variables while FAMEs content (%wt) was selected as dependent variable.

### *Free Fatty Acids determination*

FFAs were determined *via* titration using a 0.1 N KOH normalised solution and phenolphthalein as indicator in a 1:1 diethyl-ether: ethanol medium (1 g sample dissolved into 150 mL solvent).

## ANALYSIS OF RESULTS

### *Trans-esterification tests for biodiesel production*

Preliminary tests were conducted on sunflower oil to test the efficacy of the synthetized catalysts in the biodiesel production. Increasing the amount of calcium loaded from 5 to 20%wt, an increase of activity in the trans-esterification of glycerides was observed, strictly connected to the basic properties of the catalysts (Fig. 2a). When Ca loaded was 20%wt, a FAMEs content >80%wt was obtained at 100 °C after 3 h in the reaction conditions adopted (5%wt of catalyst, molar ratio methanol to oil = 15). In addition, respect to the use of CaO that was completely dissolved during the process, the catalyst was easily recovered and reused 3 times without loss of the activity. Finally, the optimization of reaction conditions were conducted by response surface methodology of a Box-Behnken factorial design of experiments (Fig. 2b). By carrying out the trans-esterification reaction at 99.5 °C for 5 h

(7.3%wt of catalyst, molar ratio methanol to oil = 15.6), a FAMEs content of >99%wt was obtained.

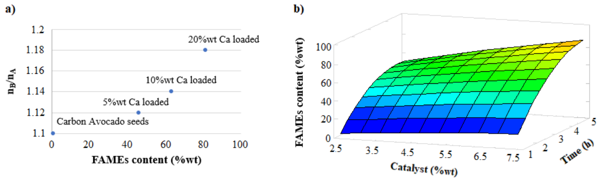

**Fig. 2.** a) Correlation between FAMEs content (%wt) and basic/acid properties ($n_B/n_A$) of the catalysts and b) Response surface plot of the effects of catalyst and reaction time at fixed temperature (80 °C) and molar ratio methanol to oil (15).

$$\text{FAMEs} = -162.71 + 4.4674X_1 + 4.5032X_2 + 7.6919X_3 + 1.8221X_4 - 0.23094X_1^2 + 0.0055167X_2^2 - 2.9705X_3^2 - 0.0051833X_4^2 - 0.139X_1X_2 + 1.156X_1X_3 + 0.0362X_1X_4 + 0.40475X_2X_3 - 0.03785X_2X_4 + 0.13169X_3X_4$$

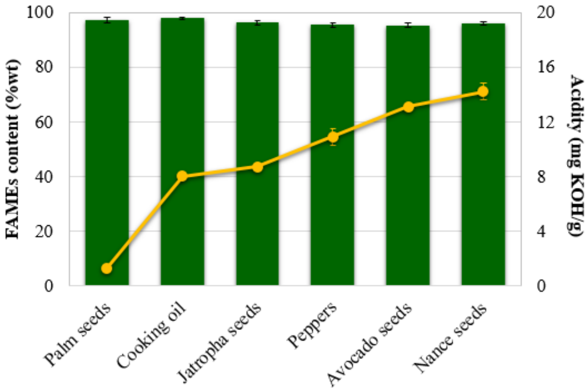

**Fig. 3.** Biodiesel production from non-edible oils by using supported catalyst with 20%wt of Ca loaded.

Finally, the optimized conditions were adopted on non-edible oils with high FFAs content (1-15 mg KOH/g). In all cases, a FAMEs content >95%wt in the product isolated was obtained, confirming the efficacy of process and the applicability of catalyst in the biodiesel production from several raw oils. However, in order to evaluate the economy of the entire process, it is necessary not only maximize the methyl esters yield but also to consider other factors such as the energy required for the recovery of methanol at the end of the process [28,29]. For this reason, further studies will be developed with the aim to optimize the overall economy of the process.

## CONCLUSION

Carbon-based calcium catalysts deriving from carbon avocado seeds were synthetized and tested in the trans-esterification reaction of sunflower oil with methanol. The catalysts were synthesized by precipitation method with calcium oxide which was homogeneously dispersed on the surface of carbonized supports. Among the different catalyst tested, supported catalyst loaded with 20%wt of Ca shows the best catalytic activity in the trans-esterification process, related to its basic properties. Finally, the catalyst was successfully tested on several non-edible oils with high FFAs content, by significantly reducing the costs required for the biodiesel production.

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# Evaluation of the synthesis routes for the preparation of heterogeneous catalysts obtained from coconut shell and its application in the biodiesel production

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The synthesis and characterization of coconut-based heterogeneous catalysts and its application in the biofuel production has been studied. Catalyst samples were prepared according to an orthogonal experimental design where the best synthesis conditions were identified. Specifically, the catalysts were obtained from a char-based support prepared from the pyrolysis of coconut endocarp, which was chemically treated with KOH and $Ca(NO_3)_2$. Catalysts were employed for the transesterification reaction of commercial safflower oil with methanol at 60 °C to produce fatty acid methyl esters (FAMEs). Surface chemistry characterization of the char support and catalysts was carried out in order to understand the impact of the synthesis route on the FAMEs yield. These catalysts showed a competitive performance for biofuel production where the maximum FAMEs yield was 90.5%.

**Key words:** Heterogeneous catalysts, transesterification, biodiesel, coconut biomass.

## INTRODUCTION

Biodiesel is an alternative energy source widely known for its friendly environmental properties. This biofuel is non-toxic and biodegradable, it also emits less pollutants including carbon dioxide emissions and contains less sulfur in comparison to fossil fuels [1]. There are four main methods to produce biodiesel that include a direct use and mixing, microemulsion, thermal cracking and transesterification [2]. In particular, the production of biodiesel via transesterification has received a significant attention during last years. This process is carried out by mixing vegetable oils or animal fats with short-chain alcohols in the presence of a catalyst to obtain fatty acid methyl esters (FAMEs) [3]. The use of a homogeneous catalyst has been associated to operational drawbacks such as the difficulty in the separation of products that are present in a homogeneous phase, including the non-reusability and high cost of the wastewater treatment system to neutralize the catalyst before being discharged [4]. On the other hand, the heterogeneous catalysts are easily separable and recyclable thus offering operational advantages for biodiesel production. They have proven to be a promising alternative with a performance comparable to that obtained for the homogeneous catalysts [5].

Heterogeneous catalysts prepared from biomasses have been studied since these feedstocks have the potential to reduce the biofuel production cost due to its abundant availability and completely renewable character. Note that this type of biomass is firstly converted into a carbonaceous material, which can be used as a catalytic support for the incorporation of active specie(s) [6]. The preparation route of the support and the modification of its surface chemistry determine the catalyst performance. Therefore, an optimization of the catalyst preparation route should be performed.

This article reports the use of coconut (*Coco nucifera*) endocarp as a precursor to synthesize a heterogeneous catalysts for the production of biodiesel. A detailed analysis of the preparation conditions has been performed to optimize the catalytic performance in the transesterification reaction of a commercial safflower oil with methanol to produce FAMEs.

## EXPERIMENTAL

For the synthesis of heterogeneous catalysts, the coconut endocarp was utilized to obtain a carbonaceous support via pyrolysis. This biomass was dried and sieved to obtain a mean particle diameter of 0.105 mm. An experimental orthogonal design was utilized to identify the impact of the catalyst preparation conditions on the percentage of FAMEs yield via the transesterification reaction of a commercial safflower oil with methanol. In particular, the lignocellulosic precursor was pyrolyzed at $N_2$ atmosphere using a tubular furnace. This carbonaceous support was submitted to a chemical activation with a solution of potassium hydroxide (KOH) with a concentration 4 M during 24 h at room temperature under constant stirring. The sample was separated from the solution and dried at 100 °C for 24 h. Then, this sample was impregnated with a solution of calcium nitrate at

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room temperature for 24 h at constant stirring. The solid material was washed and dried at 100 °C for 24 h and a final thermal treatment was performed in a tubular furnace for 2 h under $N_2$ atmosphere. The variables studied in the experimental design were: the pyrolysis temperature (600, 750 and 900 °C) and dwell time (1, 2 and 3 h) to obtain the coconut-based char, the concentration of calcium nitrate (0.1, 0.2 and 0.4 M) used to modify the surface chemistry of this char and the conditions of the thermal treatment (500, 700 and 900 °C) to active the catalyst surface.

A transesterification reaction was carried out to determine the FAMEs conversion and performance of this heterogeneous catalyst. These experiments were done with batch reactors mixing commercial safflower oil with methanol in a molar ratio of 15: 1, with 20% wt of catalyst respect to oil, at 60 °C for 8 h under constant stirring. This commercial safflower oil showed an average molecular weight of 885.47 g/mol and a fatty acid profile that included 77 % Linoleic, 13.5 % Oleic, 7.3 % Palmitic, 0.1 % Palmitoleic, 1.9 % Stearic and 0.2 % others. After the reaction, the catalyst was separated by centrifugation and the non-reacted methanol was evaporated. FAMEs amount was quantified by a Thermo Scientific Trace 1300 gas chromatograph equipped with a flame ionization detector (FID) and a TG-5 SILMS column. FAMEs amount was calculated based on a methyl heptadecanoate internal standard. The response variable of the experimental design was the percentages of FAMEs yield using the catalyst and support. Statistical analysis of the experimental design was performed using the signal-to-noise ratio (S/N) where the best conditions for the catalyst synthesis were identified with ANOVA considering the perspective "the higher, the better".

Surface chemistry of selected samples of the support and catalysts was analyzed. The crystalline structure of the samples was determined by X-ray diffraction. XRD patterns were recorded on a Malvern-PANalytical Empyrean X-Ray diffractometer using the Bragg-Brentano configuration with CuKα radiation. FTIR spectra of these samples were recorded using a Thermo Scientific Nicolet iS10 FTIR spectrophotometer in the range of 4000 to 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ using 32 scans. Samples were dispersed in KBr reactive to be measured as pellets.

## RESULTS

Results of the transesterification reaction of safflower oil using the heterogeneous catalysts showed a FAMEs yield from 0.4 to 90.5%. In particular, the best catalyst was obtained from the coconut endocarp pyrolysis at 600 ° C for 1 h, impregnated with KOH and $Ca(NO_3)_2$ 0.1 M and thermally treated at 700 ° C. This material showed the highest FAMEs yield (i.e., 90.5 %). Herein, it is important to remark that the FAMEs yield obtained with the char support (i.e., with no further modification) was 0.25 %, which indicated that the activation stage with KOH and $Ca(NO_3)_2$ has a significant impact on the catalytic properties. On the other hand, it was identified that the final thermal treatment after the nitrate calcium impregnation played an important role to improve the catalyst performance. Some samples obtained after the KOH treatment also showed competitive catalytic properties. These results indicated the relevance of synthesis route conditions in the properties of heterogeneous catalysts for biodiesel production.

Figure 1 shows the results of the signal-to-noise ratio where the impact of tested catalyst synthesis variables on the transesterification reaction can be observed. Statistical analysis clearly demonstrated that the catalyst properties were totally dependent on the conditions used in the synthesis. ANOVA analysis indicated that the pyrolysis conditions of the catalytic support were the variables with the highest impact in the synthesis process. Low FAMEs yields of the catalyst could be due to the degradation of the surface functionalities of the char support due to the increment of the pyrolysis temperature and time [7]. These results confirmed that it is paramount to analyze the synthesis procedure and its operating conditions in order to select the route that provides the best properties to the support and catalyst [8,9].

XRD diffractograms and FTIR spectra are shown in Figure 2. XRD spectrum of the char support showed the peaks corresponding to an ordered carbon structure in a graphitic form with low crystallinity [10-12]. The crystallinity of modified coconut chars showed a slight decrement after the treatment with KOH. This change could be attributed to the presence of potassium on the solid surface. This support modified with KOH and $Ca(NO_3)_2$ showed peaks at 28, 36, 39 and 48 ° related to calcium carbonate according to the database HighScore Plus. These results were similar to those reported for materials prepared by other authors [13]. FTIR spectra of char and catalyst samples contained some characteristic bands of the lignocellulosic precursor, see Figure 2b. Specifically, the stretching vibration of the OH- appeared in the region 3600-3000 $cm^{-1}$, which can be associated to phenols and alcohols in cellulose, hemicellulose and lignin [14]. The absorption bands located in the region of 2916-2840 and 1504-1236

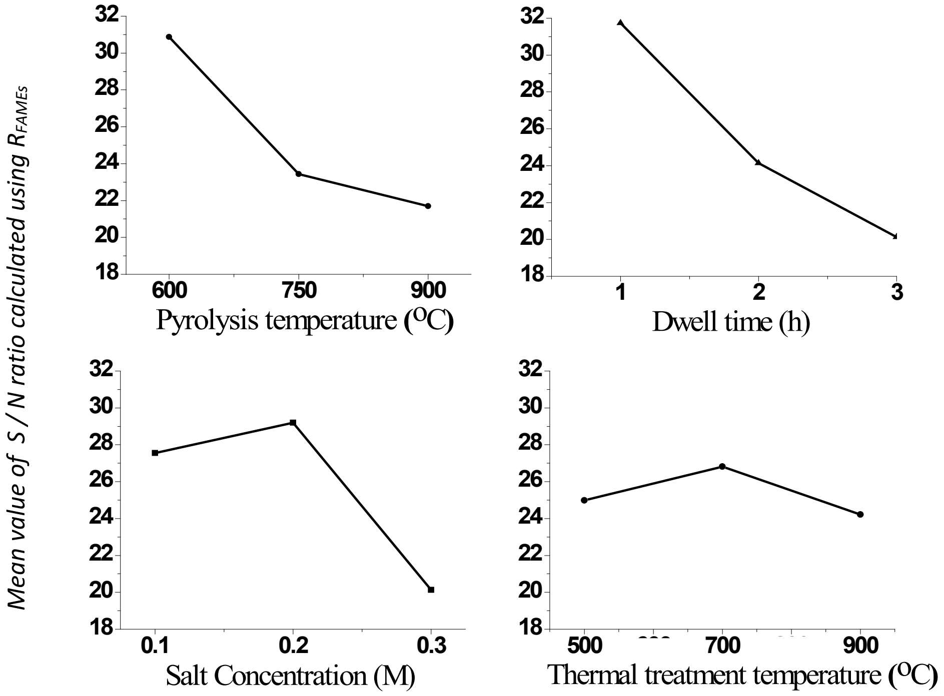

**Fig. 1.** Results of S/N ratio for the variables analyzed in the preparation of heterogeneous catalysts obtained from coconut biomass.

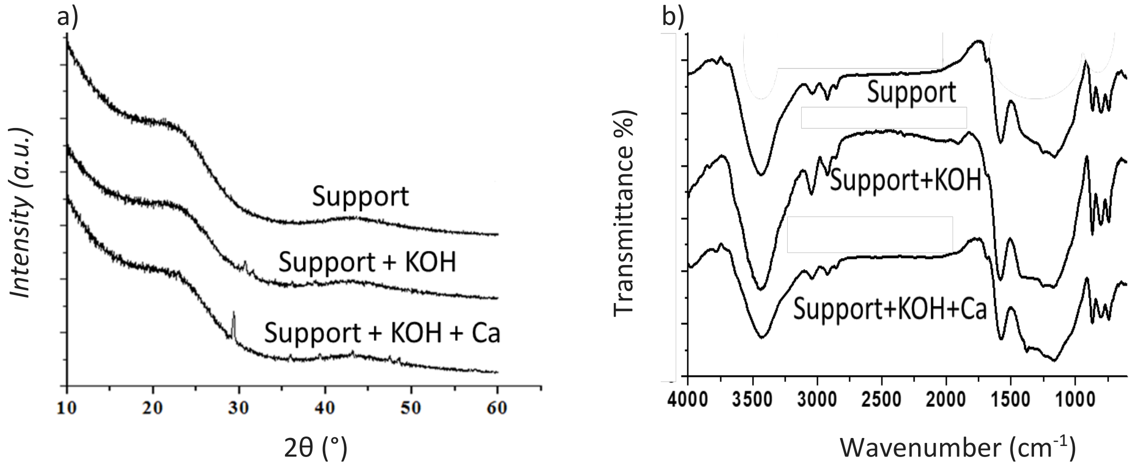

**Fig. 2.** a) XRD diffractograms and b) FTIR spectra of the coconut-based heterogeneous catalysts.

cm$^{-1}$ corresponded to CH of aliphatic groups. The bands at 1734 and 1603 cm$^{-1}$ were assigned to the C=O vibration in the form of ketone or carboxylic acid, while the bands at 1520 and 890 cm$^{-1}$ indicated the presence of structural C=C of aromatic rings [11]. Finally, the band at 1160 cm$^{-1}$ can be attributed to C-O vibrations indicating the presence of acids, alcohols, ethers and esters. Note that the FTIR spectrum of the coconut char modified with KOH did not show significant changes in the absorption bands. For the char treated with KOH and Ca, the band at 3400 cm$^{-1}$ reduced and broadened, while the band at 1400 cm$^{-1}$ increased its intensity. These changes could be related to the presence of calcium as Ca-OH and Ca-O thus suggesting the coordination of this metal with hydroxyl and carboxylic groups [15].

## CONCLUSION

The preparation of catalytic supports obtained from coconut endocarp and its performance in FAMEs production was analyzed and discussed. The highest FAMEs yield was obtained with the catalyst prepared via pyrolysis of coconut biomass at 600 °C for 1 h, modified with KOH and $Ca(NO_3)_2$ 0.1 M and thermally treated at 750 °C. This catalyst showed a 90.5 % FAMEs yield in a transesterification reaction of commercial safflower oil with methanol at 60 °C. These values were competitive and even higher than those reported in the literature for other heterogeneous catalysts. Statistical analysis allowed to identify the variables with a significant impact on the preparation of this heterogeneous catalyst, where the pyrolysis time and temperature showed the greatest influence on the properties of coconut-based catalyst. Finally, it was concluded that the selection of the synthesis route was paramount to obtain a reliable heterogeneous catalyst with competitive properties to be used in the production of biodiesel.

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# Integrated facility for the use of oranges as a source for power, chemicals and juice

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In this work, we evaluate the integration opportunities within the juice production industry. The process begins with the oranges reception and juice production. The peel is further processed for the production of limonene. Next, the waste is digested to provide for the thermal and electrical energy required for the facility using a gas turbine. The process results in a highly integrated facility that allows implementing the concept of circular economy within the fruit industry. However, even though the facility is profitable, with a benefit after taxes of 56%, if higher added value products are to be produced such as limonene, the utilities required by the process cannot be produced from the residues affecting the sustainability of the process.

**Key words:** Biogas, anaerobic digestion, manure, power production, mathematical optimization

## INTRODUCTION

The concept of circular economy is a current trends towards a more efficient production system where waste is reused and recycled as raw material. In particular, the food industry is characterized by the production of large amounts of waste in the processing of the raw material. To improve the efficiency of these plants integrated design as a chemical complex will allow reusing the waste into valuable products and energy [1]. In the case of the fruit industry, in the production of juice together with the main product waste is produced [2]. This residue is also part of the municipal solid waste that is being treated for energy production. But before that final use, it is important to notice that the peels contain added value products. In particular, citrus peels contain compounds such as pectin, limonene [3-5] that can and should be recovered before waste treatment. Anaerobic digestion (AD) has been deemed as an efficient process to treat waste [6]. The main products from the AD of waste consist of biogas, composed of $CO_2$ and $CH_4$ that can be an interesting source for chemicals via dry reforming [7], power [8] and a digestate rich in nutrients that can be recovered in different forms [9]. Therefore, a facility for the production of juice can become a highly integrated chemical complex.

So far, the evaluation of the use and products from the fruit industry has been addressed separately. AD of waste has been studied towards evaluating the yield to biogas [10]. The recovery of added value products has been experimentally studied as well as the pectin recovery [3]. Only lately the last part of the process, from the waste towards limonene and p-Cymene including the possible use of the waste to produce power via gasification has been considered [11] However, the large water content and the need to define the synergies for the entire process, from the fruit towards the multiproduct suggest the use of a systematic approach

In this work we develop an integrated facility for the production of juices, limonene and power from oranges using a mathematical optimization formulation in an integrated facility that uses the exhaust gases from the gas turbine to produce steam that eventually produces power in a steam turbine. The rest of the paper is organized as follows: in section 2 we provide a brief description of the process; in section 3, the different units are described and the modelling assumptions are presented; in section 4, the results of the optimal operation of the facility are shown together with an economic evaluation; and finally, in section 5, we draw some conclusions.

## PROCESS DESCRIPTION

The process consists of fruit processing, limonene extraction, biogas production, biogas purification (biomethane generation), gas turbine (Brayton cycle) and the use of the flue gas to provide the energy within the process, see Figure 1.

Sweet oranges are washed using 2 L of water per kg of oranges and squeezed. It is a mechanical step where juice and the peels are obtained separately. The pulp can be left in the juice or removed by filtration. This pulp, if removed, can be used within the peel residue in the AD step. The juice must be thermally treated in two steps to remove microorganisms and denaturalize enzymes. The first stage consists of heating up the juice 368 K for 10-30 s. After that, and before bottling it up, the juice is heated up again for 15 s ant 368 K [12] The peels constitute 40-60% of the orange.

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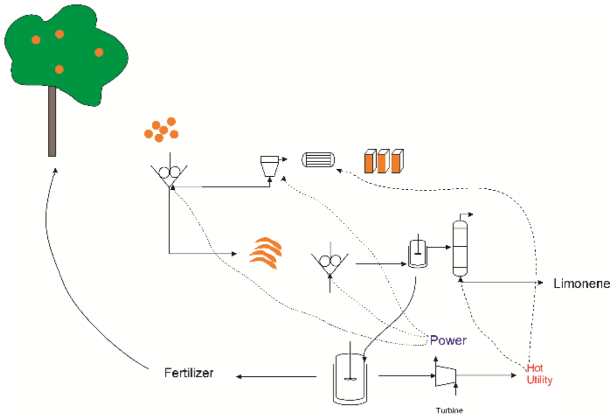

**Fig. 1.** Flowsheet for the integrated production of juices, limonene and power.

Limonene, d-limonene, can be recovered from the peel by extraction. The peel must be milled to increase the surface area. Next, n-hexane is used to extract it, so that 0.55 $dm^3$ are recovered per $m^3$ of hexane used [13]. The peel residue is separated by filtration. Finally, the hexane is recovered by distillation. The distillation column works under vacuum to avoid decomposition of the limonene.

The peel residue is fed to a bioreactor where it is anaerobically digested to produce biogas and a decomposed substrate (digestate). The biogas is composed of methane, carbon dioxide, nitrogen, hydrogen sulphide, ammonia and moisture. It is sent to purification to remove first the $H_2S$ in a fixed-bed reactor and later the $CO_2$ (and traces of $NH_3$), using Pressure Swing Adsorption (PSA). Once the biogas is mainly methane, it is used in the Brayton cycle, where the biomethane is compressed, burned with air and expanded in a turbine, generating power.

The digestate can be processed to recover the nutrients following different technologies. Struvite production is recommended if it is to be transported long distances, but if the facility is allocated close to the harvesting point of the oranges it can be just recovered as a cake.

## MODELLING ISSUES

The models for each of the units involved in the process, are formulated using mass and energy balances, experimental yields, thermodynamics, chemical and vapor-liquid equilibria to evaluate their performance. Details of the modelling approach can be seen in [14]. The process model is written in terms of total mass flows, component mass flows, component mass fractions, temperatures and pressures of the streams in the network. Table 1 summarizes the modelling approach for each of the units involved in the process.

**Table 1**. Summary of modelling approach for the integrated facility

| Unit | Modelling approach |
|---|---|
| Washing | M&E Balances<br>Exp. Data |
| Grinding | M&E Balances<br>Exp. Data on Power |
| Juice treatment | M&E Balances<br>Exp. Data on T and time |
| Peel milling | M&E Balances<br>Exp. Data on power [18] |
| Limonene extraction | M&E Balances<br>Exp. Data on extraction [13] |
| n-Hexane recovery | Fenske eqs<br>M&E Balances [16] |
| AD | M&E Balances [8] |
| $H_2S$ Removal | M&E Balances [8] |
| $NH_3$ /$CO_2$ Removal | M&E Balances [8] |
| Brayton cycle | Thermodynamics<br>M&E Balances [8] |

The facility is modelled and optimized for the optimal operation producing the power and hot utilities required for the operation of the entire integrated process maximizing the profit obtained from the juice, the limonene and minimizing the input of utilities. The orange peels after pretreatment for limonene recovery shows the composition given by Table 2.

**Table 2.** Orange peels composition for digestion [17]

| | |
|---|---|
| Vbiogas ($m^3$/kg) | 0.35 |
| WDM | 0.21 |
| WVS | 0.85 |
| WN | 0.002 |
| WNorg | 0.010 |
| WP | 0.002 |
| WK | 0.009 |
| RCN | 15 |

WDM: Dry matter weight percentage; WVS: Volatile weight percentage; WC: Carbon weight percentage; WN Inorganic nitrogen weight percentage; WNorg: organic nitrogen weight percentage; WP: Phosphorous weight percentage; WK: Potassium weight percentage; RCN: Carbon to Nitrogen ratio

The investment cost of the facility is based on the factorial method [15] where the cost for the different units has been estimated based on [16] updating of the units when required. For the production cost, again, the factorial method is used. The cost of the oranges for juice is taken to be 0.1 €/kg [19], the cost of steam 0.019 €/kg [20] and that of cooling water 0.00057 €/kg [21] while the fresh hexane is taken to be 1.5 €/kg [22]

## RESULTS

The mass and energy balances result in the values reported in Table 3 where the major ratios are reported for a facility that processes 5kg/s of oranges. In the results it is possible to see that the facility can provide its own power for running the grinding, milling and centrifuges. However, the extraction of limonene requires a large amount of energy for the recovery of the solvent, resulting in a deficit that must the provided from external resources. The thermal energy available is enough for the production of the juice and its pasteurization, though, but not enough for the facility to be selfsustained. Fertilizers and limonene are also produced a part from the juice that can be further used to obtain credit and/or reduce the needs of nutrients in the following growing period.

**Table 3**. Major results of the flowsheet operation

| | |
|---|---|
| Production of Juice | 0.5 kg /kg of Oranges |
| Peels | 0.5 kg /kg of Oranges |
| Hexane added | 0.1209 kg/kg of peel |
| Biogas produced | 0.0464 kg /kg of peel |
| Fertilizer | 0.1696 kg dry /kg of peel |
| NPK index | 0.17/0.1/0.24 |
| Limonene produced | 0.00924 kg /kg Peel |
| Air excess (Gas turbine) | 21% |
| Power produced | 280 $kJ_e$/kg of Peel |
| Power consumed | 3.6 $kJ_e$ /kg Peel (Milling) [18] |
| Thermal Energy available | 350 $kJ_t$/kg of Peel |
| Thermal Energy required | 150 kJ / kg peel ( Pasteurization)<br>5400 kJ /kg peel ( Solvent recovery) |

The economic analysis can be summarized in the following values. For a facility that processes 5kg/s of oranges (155kt/yr), the integrated facility investment cost adds up to 59 M€ with a production cost of 41.7 M€/yr to produce 77760 $m^3$ of juice per year, 519 ton/yr of limonene and 5.83 GWh of power. The facility requires steam, around 6kg/s and cooling water, 304 kg/s. the profitability of the facility highly depends on the juice cost per litter. For the products prices of 1 €/L of juice, the limonene costs at 15 €/kg and 0.06 €/kWh obtained from the excess of power, without considering any credit out of the digestate, the benefit after taxes is 56%. Out of the digestate additional income can be obtained depending on the market and its form at the processing cost of each alternative [9] unless is internally used for the next harvest in which case it is expected to decrease the price of the oranges. Figure 2 shows the breakdown of the production cost (a), and the investment cost (b). almost 40% of the production cost goes to the raw material, another 34% corresponds to chemicals while utilities and equipment amortization reach 10% each. In terms of the investment, the section for the production of limonene represents 50% of the cost of the facility, while that of the waste treatment adds up to 30%. Juice production and pasteurization reaches 10%, since it includes the HXs.

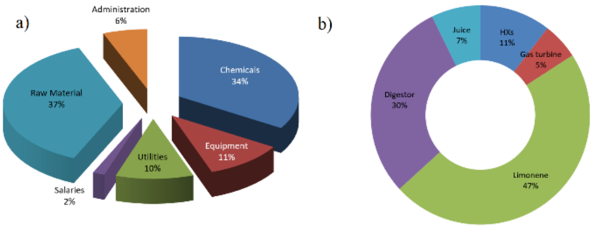

**Fig. 2.** Breakdown of the investment (a) and production costs (b).

## CONCLUSION

In this work the concept of an integrated facility for the production of juices, limonene and power from oranges is evaluated using a mathematical optimization formulation. Waste is used to produce utilities for the operation of the plant, power and heat, required by the facility including juice pasteurization and limonene extraction.

The facility can provide its own electricity, as well as thermal energy for juice production, but the production of limonene requires a large amount of energy for solvent recovery. The process is economically interesting, with a benefit after taxes of 56%, but it is highly energy intense. More efficient limonene production paths and recovery technologies must be addressed for a cleaner production process. Furthermore, scale up studies are required to evaluate the effect of the exploitation size on its economics.

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# *Valorization of biomass waste streams*

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# Mexican biomass wastes: valorization for potential application in bioenergy

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The conversion of biomass into biofuels and biochemicals, represent a useful way to reduce the use of fossil fuels and maintain a sustainable energy production. In this context, Mexico is a country with a wide variety of exploitable agricultural resources that can be used to support its economic growth. In this study, several Mexican biomass wastes (avocado seeds, palm seeds, peppers, flamboyant fruit, jatropha seeds, coconut shells and nance seeds) were analyzed and fully characterized, in order to obtain a complete exploitation of their energy potential.

**Key words:** Bioenergy, valorization, waste biomass, biodiesel, renewable energy, biorefinery

## INTRODUCTION

As a result of population growth combined with the gradual depletion of fossil fuels and the environmental problems associated with their use, an increase of energy demand worldwide will be expected [1,2].

For this reason, the use of alternative renewable energy is necessary in order to maintain a sustainable energy production. The conversion of biomass into of biofuels and biobased chemicals represent a useful way to mitigate the global warming and diversify the energy sources [3-5]. Biomass is a renewable resource that can be obtained from agricultural residues [6], industrial and animal wastes [7] and sludge from water treatment plants [8,9]. It is more evenly distributed over the Earth's surface respect to fossil fuels such as oil, coal and natural gas and it can be developed by using environmentally friendly technologies. Fuels derived from biomass are biodegradable, renewable, oxygenated with higher combustion efficiency and lower sulphur and aromatics content [10]. In addition, biomass is a valuable source of chemicals, pharmaceuticals and food additives [11]. Energy from biomass, also known as bioenergy, it was identified as the highest potential renewable energy in Mexico (from 2635 to 3771 PJ/year), with this research topic that was increasingly explored by Mexican academia. Furthermore, the Mexican General Law for Climate Change aims to generate 35% of its energy needs from renewable energy by 2024 [12]. The choice of raw materials that can be used in bioenergy processes depends on their physico-chemical properties and availability. In this sense, it is necessary to identify biomasses with potential to be used for the production of bioenergy. In this work, a study of the valorization of the principal Mexican biomass wastes was performed, in order to identify their potential applications for the production of fuels and value-added chemicals. In detail, avocado seeds, palm seeds, peppers, flamboyant fruit, jatropha seeds, coconut shells and nance seeds were analyzed and fully characterized, by evaluating the possible industrial applications.

## MATERIALS AND METHODS

### *Reagents and Instruments*

All chemical reagents used in this work were of analytical grade and were used directly without further purification or treatment. Avocado seeds (Persea Americana), peppers (Hungarian yellow and red variety) and seeds of palm (Palma de Coroco), jatropha (Jatropha Curcas), nance (Byrsonima crassifolia) were purchased from a local market of Aguascalientes (Mexico).

Fatty Acid Methyl Esters were identified by gas chromatography-mass spectroscopy (GC-MS) by using a Perking Elmer Clarus 500 equipped with a Clarus spectrometer. Quantitative determinations were carried out with a Varian 3800 GC-FID. Both instruments were configured for cold on-column injections with a HP-5MS capillary column (30 m; Ø 0.32 mm; 0.25 μm film).

### *Determination of Total Solids and Ashes*

Total solids (TS) were determined according to the ISO 11465 method [13]. 10 g of biomass were placed in an oven at 105 °C for 24 h, until a constant weight was obtained. Then, the dried samples were heated in a muffle furnace at 550 °C for 3 h and ashes content was also determined. The results were expressed as weight percentage (%wt) of residual

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solids obtained after each thermal treatment, respect to the starting material.

### *Extraction of raw oils*

In a Falcon tube of 50 mL, 5 g of dried biomass were placed with 20 mL of hexane. The system was closed and shaken for 10 min. At the end of process, a biphasic system was obtained: i) an organic phase in which raw oil was dissolved and ii) a lower phase of wet solid. Then, the organic phase was then separated from the residual biomass and recovered. The extraction procedure was repeated other three times with all organic fractions that were collected together. Finally, the solvent was evaporated under nitrogen flow and raw oil was weighed and analyzed in terms of Acid Value, Fatty Acid profile and Average Molecular Weight.

### *Determination of Acid Value*

In a flask of 250 mL, 1 g of raw oil was placed with 100 mL of diethyl-ether:ethanol solution (1:1 v/v) in presence of phenolphthalein as indicator. Then, the organic mixture was titrated with 0.1 N KOH solution to the phenolphthalein endpoint. The results were expressed as milligrams of KOH required to neutralize 1 g of the raw grease (mg KOH/g).

### *Fatty Acid profile and determination of Average Molecular Weight (AMW)*

20 mg of raw oil and 2 mL of toluene, methanol and concentrated $H_2SO_4$ (2:2:0.01 v/v/v) were placed in a glass tube of 10 mL. The system was closed and heated at 70 °C for 5 h. Then, 1 mL of 1000 ppm methyl heptadecanoate toluene solution was added as internal standard and the resultant solution was analyzed. Average Molecular Weight (*AMW*) was determined according to the following equation:

$$AMW = \frac{\Sigma A_i\, MW_i}{\Sigma A_i} \qquad (1)$$

where *Ai* and *MWi* are the area and molecular weight of FFAs, respectively.

### *Determination of Structural Carbohydrates and Lignin*

NREL method for "Determination of Structural Carbohydrates and Lignin in Biomass" was partially adapted and applied on residual dried samples in order to evaluate the content and the composition of simple and structural complex sugars [14]. In detail, by using different hydrolytic solutions, with increasing acidic strength, it was possible to identify and quantify separately simple sugars, from hemicelluloses, starches and pectinic sugars, from cellulosic component. 2 g of residual dried solids (after extraction procedure with hexane) were suspended into 100 mL of 4%wt $H_2SO_4$ and stirred at room temperature for 1 h. 2 mL of this suspension was filtered, opportunely diluted if necessary and analyzed for determination of free sugars (glucose, fructose and saccarose were complexively found). Then, the suspension was refluxed for 4 h. The resultant cooled suspension was filtered: limpid solution was brought again to 100 mL of mQ (milli-Q) water, diluted if necessary and analyzed, by allowing hemicelluloses, starches and pectinic sugars to be determined. On the other side, the solid filtered from the suspension was washed with over 100 mL of mQ water, dried at 105 °C for 24 h and weighted. 200 mg of this residue was suspended and kept under agitation at 4 °C for 24 h in 3 mL of 72% $H_2SO_4$. Then, it was brought to 100 mL with mQ water, the resulting solution was transferred into a 250 mL glass balloon and kept under reflux for 4 h. The suspension was cooled and filtered on a filter previously prepared and weighted. The clear solution was diluted if necessary and analyzed for sugars determinations. Finally, filtered solids were abundantly washed with mQ water and dried at 105 °C for 24 h and weighted. Insoluble lignin was calculated by the difference between this weight and the respective ashes obtained after putting the same filtering crucible into an oven at 550 °C for 3 h.

## ANALYSIS OF RESULTS

### *Analysis of the chemical composition of Mexican biomass wastes*

In Figure 1 were reported the results obtained from the chemical characterization of Mexican biomass wastes. Considering the different water content and consequently the respective TS values, an oily component was observed in most of the samples analyzed. In particular, seeds of palm and jatropha present the highest oil content range between 48-50% and 25-27%, respectively. On the other hand, structural carbohydrates such as hemicellulose, starch, pectin sugars cellulose are present in high amounts in biomass like avocado seeds and peppers, until to reach 44-50% of the overall biomass. Finally, in the case of flamboyant fruit, nance seeds and coconut shells, lignin turns out to be the main component (50-75%wt). Based on these data, different strategies can be adopted, in order to obtain a complete exploitation of their energy potential.

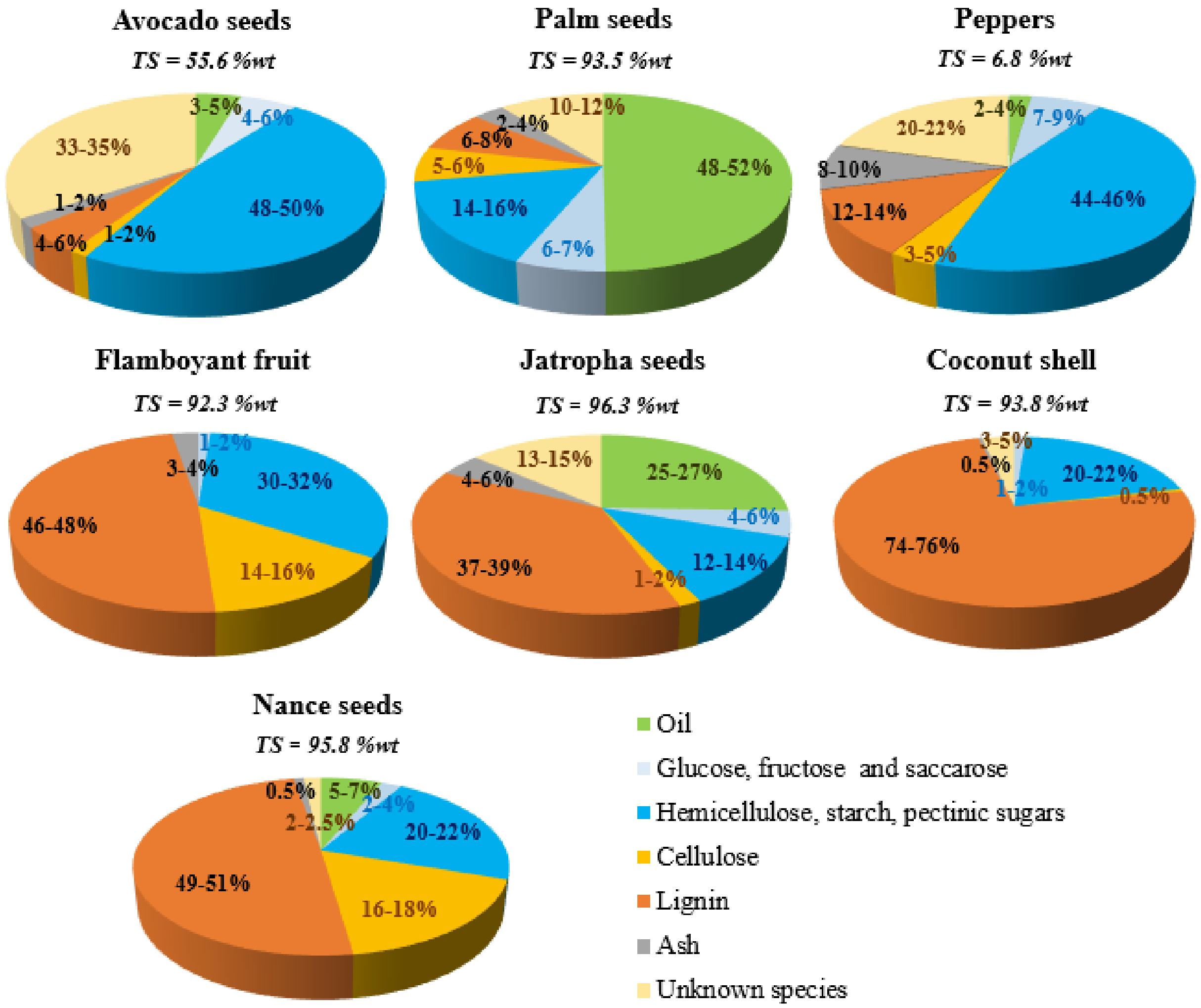
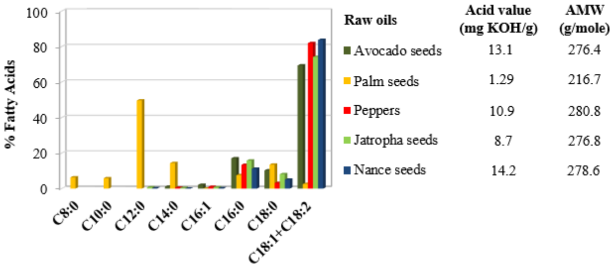


**Fig. 1.** Chemical composition of several Mexican biomass wastes.

*Biodiesel production from non-edible oils*

The use of non-edible oils as feedstock for biodiesel production represent a useful way to reduce the manufacturing costs and does not compete with food production. Biodiesel or Fatty Acid Methyl Esters (FAMEs) is typically produced by trans-esterification of vegetable oils and animal fats with methanol in presence of homogeneous basic catalysts [15]. The presence of a large amount of Free Fatty Acids (FFAs) leads to the formation of soaps with the consequent deactivation of the catalyst and difficulty in the product separation [16]. For this reason, a preliminary study of the chemical composition of the oils extracted was carried out in terms of Acidity, Fatty Acids profile and AMW. The results are reported in Figure 2.



| Raw oils | Acid value (mg KOH/g) | AMW (g/mole) |
|---|---|---|
| Avocado seeds | 13.1 | 276.4 |
| Palm seeds | 1.29 | 216.7 |
| Peppers | 10.9 | 280.8 |
| Jatropha seeds | 8.7 | 276.8 |
| Nance seeds | 14.2 | 278.6 |

**Fig. 2.** Comparison of chemical characteristics of raw oils extracted from Mexican biomass wastes.

Raw oils contain high amounts of FFAs between 1 and 15 mg KOH/g with oleic and linoleic acids which represent the main component (>70%). In this case, a two-step approach can be efficiently adopted for the biodiesel production, in which metal hydrated salts are firstly used for the esterification of FFAs, followed by trans-esterification of lipids with sodium or potassium hydroxide [7]. Biodiesel obtained was found to EN 14214 specifications.

### *Direct conversion of structural carbohydrates into biofuels and chemicals*

The conversion of structural carbohydrates (simple sugars as glucose and fructose, hemicellulose, cellulose and starch) into chemicals represents an alternative method for the synthesis of new molecules with added value and biofuels [17,18]. Ethyl levulinate is a promising platform molecule obtainable from simple and complex carbohydrates, which can be used for the production of biochemicals such as herbicides, plasticizers, resins, solvents, as well as biofuels [19]. A combined Lewis-Brønsted acid ethanolysis by using sulphuric acid and aluminum chloride hexahydrate as catalysts (Figure 3), allows the synthesis of Ethyl Levulinate in a single step from several kind of biomass in high yields with a low environmental impact [20].

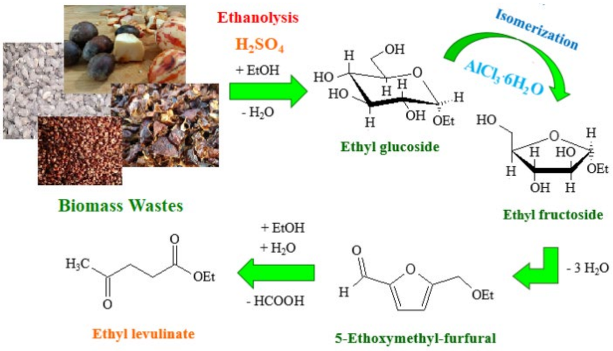


**Fig. 3.** Biodiesel production from non-edible oils by using supported catalyst with 20%wt of Ca loaded.

### *Synthesis of bioadsorbents for the removal of heavy metals from wastewater*

Activated carbon has been widely used as adsorbent for the removal of heavy metals from wastewater [21]. In recent years, the necessity of safe and economical methods for the treatment of contaminated waters led to the production of low-cost alternatives respect to the commercial product. Biomass wastes can be efficiently used as precursors for the preparation of bioadsorbents and employed to the removal of heavy metals like lead, chromium, mercury, cadmium and arsenic [22, 23].

## CONCLUSION

In this work, a detailed physico-chemical characterization of several Mexican biomass wastes biomass was carried out, in order to determine their potential applications as renewable energy sources. Different technologies can be adopted for a complete valorization of these resources, according to the principles of the green economy.

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# Assessing of potential of Aronia Berries residue after juice extraction as a feedstock for platform molecules production

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The recovery of fruit industrial waste for the production of added value compounds is a topic of great importance for the application of circular economy principle. Pomace waste coming from Aronia melanocarpa industrial utilization is an interesting source of bioactive compounds such as dietary fibre, pectins, cell wall polysaccharides, vitamins, polyphenols, phospholipides. The extraction of bioactive substances has been carried out through an enzymatic approach followed by ultrafiltration technique. The obtained residues have been collected and characterized. The obtained data revealed the presence of free and complex sugars such as glucose, fructose, sorbitol, EHS and cellulose that could be considered starting feedstock for platform molecule production.

**Key words:** Aronia Melanocarpa, waste characterization, waste valorisation, carbohydrates conversion

## INTRODUCTION

Industrial ecology concepts such as circular economy are considered leading principle for eco-innovation, aiming at “zero waste economy” in which waste are used as raw material for new products and applications. The large amount of waste produced by the food industry, have the potential to be reused into other production systems, trough e.g. biorefineries. Fruit pomace is a by-product of fruit industry that should be included in the circular economy concept. According to the literature, fruit pomace is a valuable source of nutritious components, such as proteins, fat as well as bioactive compounds such as dietary fibre, pectins, cell wall polysaccharides, vitamins, polyphenols, phospholipids [1]. In fact, during the manufacturing process of fruit juices, most of the above-mentioned components does not transfer to the juice and remains in pomace [2]. On the other hand, pomace may be a valuable source of interesting products such as simple and complex sugars, fats and organic acids that could be considered feedstock for platform molecules.

In the present work the case of Black chokeberry (Aronia Melanocarpa) has been studied. Usually, it is subjected to industrial processing for the production of juices or jams as it is a very rich source of substances exerting a beneficial impact on health, including mainly polyphenols (proanthocyanidins, anthocyanins, flavonoids, and phenolic acids). During the industrial process of extraction juice, a significant amount of pomace is produced (up to 16% of the mass of fruit being subject to processing) [3].

Herein we describe a process for the extraction of valuable compounds present in Aronia pomace and their potential conversion into added value compounds through thermo-chemical techniques.

## EXPERIMENTAL

All chemical reagents were of analytical reagent grade and were used directly without further purification or treatment. Chokeberry (*Aronia Melanocarpa*) pomace was picked up at the juice producer Elkærholm (Egtved, Denmark).

Sugars were determined using a GS50 chromatography system (Dionex-Thermo Fisher Scientific, Sunnyvale, CA, USA) equipped with an AS50 autosampler, an ED50 pulsed amperometric detector using a gold electrode and a Carbopac PA10 analytical column (250 mm, 4 mm; Dionex).

*Extraction of bioactive compounds:* 60 g of chokeberry pomace were mixed with 3 L of acidified water (50 mM citric acid, pH 2.3) and homogenized with an ultra-turrax (30 min, 9600 rpm, ~323 K). The pectinase- based enzyme formulation Fruktozym® Flash-C (Erbslöeh Geissenheim AG, Germany) was added to the reaction mixture at a dosage of 2 mL $kg^{-1}$. The extraction continued for other 30 minutes at 323 K based on a previously optimized method. After this, the ultrafiltration process to collect the extract

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started using a tubular single-channel 25 kDa ceramic membrane (batch nr. 267449) from Atech Innovations Gmbh (Gladbeck, Germany). Two residues were collected at the end of the process (retentate and permeate).

*Chemical characterisation of the extraction residues:* Upon the extraction protocol for bioactive molecules recovery, the resulting residues (retentate and permeate) were acidified using $H_2SO_4$ (4%) in order to stop the enzymatic activity. The acidified matrixes were kept under stirring at room temperature for 1 h. Then, 2 mL were filtered, diluted and analysed through IC-HPAD to determine free simple soluble sugars. [4]

The remaining part of the suspension was refluxed for 2 h. The obtained suspension was filtered by Whatman filter, and 2 mL of the filtered solution were diluted to a total volume of 200 mL with distilled water. The obtained solution was then analysed at IC-HPAD for sugar determination (hemicelluloses, starches and sugars released from pectins). [4]

Solid recovered from the filter were washed with distilled water and dried at 378 K for 24 h. Then, it was transferred into a glass tube and left in suspension with 5 mL of 80% $H_2SO_4$ at 277 K for 24 h. At the end, it was again refluxed for 2 h in an overall volume of 100 mL milli-Q water. The resulting suspension was cooled and filtered on a filtering crucible previously prepared and weighed. The filtered solution was diluted and analysed for sugar determination (cellulose). [4]

## RESULTS AND DISCUSIONS

Aronia Melanocarpa is a very rich source of numerous substances [5]. Among that, the most important are the phenolic compounds (proanthocyanidins, anthocyanins, flavonoids, and phenolic acids), possessing antioxidative, anti-inflammatory, antiviral, anticancer, antiathero-sclerotic, hypotensive, antiplatelet, and antidiabetic properties.

The enzymatic degradation of plant cell-wall polysaccharides has been reported to facilitate the release of bioactive compounds into the extraction media [6] and to increase the filtration rate [7]. The enzyme products used for this purpose are typically pectinases, cellulases or proteases in single enzyme preparations or in blends.

In this work the isolation of bioactive compounds from plant waste has been carried out by using an enzymatic approach coupled to ultrafiltration: the extraction mixture obtained from enzymatic process (feed) was pumped to the membrane, the retentate was recirculated to the feed tank while the permeate (product) was collected separately. The ultrafiltration process was performed for 82 minutes, in which 677 g of permeate were collected. The permeate stream accounts for 22 % of the starting material and is rich in water-soluble small molecules. On the other hand, the retentate stream (88%) contains the particulate matter corresponding to the residual cell wall polysaccharide matrix.

The two streams obtained from such protocol, namely permeate and retentate, have been collected and characterized in order to check the presence of sugars, fat and lipids. The obtained results are summarized in Table 1.

**Table 1.** Chemical composition of the ultrafiltration streams

| Constituents | | Feed | Retentate | Permeate |
|---|---|---|---|---|
| Free Sugars (ppm) | Sorbitol | 700 | 680 | 730 |
| | Glucose | 605 | 601 | 610 |
| | Fructose | 620 | 597 | 634 |
| EHS (ppm) | Hemi-celluloses<br>Starches<br>Pectins | 257 | 557 | 6 |
| Cellulose (ppm) | | 85 | 293 | 6 |

The obtained data reveal the presence of free sugars, mainly sorbitol, glucose and fructose, that are equally distributed between the permeate and retentate and carbohydrates such as EHS (revealed by the presence of arabinose, galactose, glucose, xylose and mannose that are the main constituents of hemicelluloses, starch and pectin) and cellulose. Both the last two remain mainly in the retentate during the ultrafiltration process. The presence of such species increases the potential utilization of Aronia waste since they are all potential starting feedstock for added value products (Fig. 1).

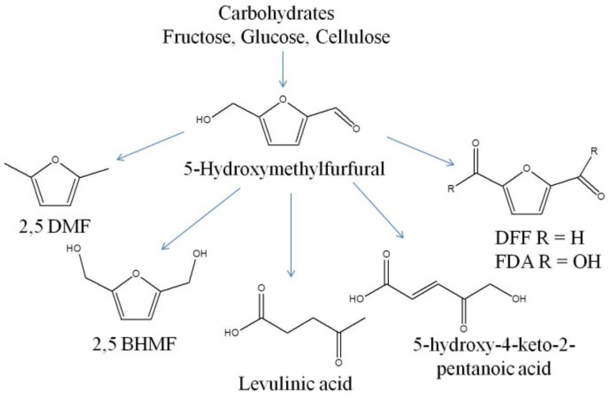

**Fig. 1.** Conversion of carbohydrates into added value products.

Of particular interest is the presence of sorbitol that is one of the component of the *top added value* *chemicals from biomasses* list: basing on data from the literature, among a series of fruits and berries, Aronia contains the highest concentration of sorbitol [8]. Sorbitol could be used as well (toothpaste, Confections and food, Ascorbic acid, Industrial surfactants, Pharmaceuticals) or as a platform chemical. The main uses are as source for ethylene glycol (EG), propylene glycol (PG), 1, 2-propanediol (1, 2-PDO), glycerol and lactic acid production [9]. Sorbitol is also suggested for the application of Aqueous Phase Reforming (APR) for the production of $H_2$ thus providing hydrogen needed in many other biorefining operations, such as hydrodeoxygenation [10]. Moreover sorbitol is a key intermediate in the production of liquid straight-chain alkanes such as n-hexane, n-pentane and their isomers which are also commonly known as gasoline alkanes [11] (Fig. 2).

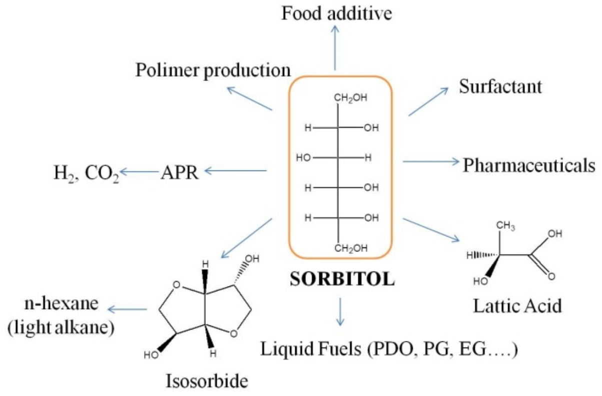

**Fig. 2.** Potential use of sorbitol.

## CONCLUSION

Aronia Melanocarpa pomace coming from the industrial juice production has been studied in order to check the presence of bioactive molecules and other compounds to be used as feedstock for valuable product. Upon extraction of bioactive compounds by utrafiltration, the presence of interesting amount of sugars has been detected namely glucose, fructose, sorbitol, EHS and Cellulose. Potential pathway for the valorization of such compounds have been elucidated.

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# Optimization of the Anthocyanins extraction process from Aronia berries pomace

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The extraction of anthocyanins from *Aronia melanocarpa* pomace from juice production was studied in the context of waste minimization and biomass valorization. The influence of temperature, solvent concentration and solid to solvent ratio was investigated. The concentration achieved with extraction was quantified by HPLC. The highest concentration of anthocyanin observed was 794.16 mg/l at 50°C, using a concentration of citric acid of 1.5 wt% and a 1:20 solid to solvent ratio.

**Keywords:** *Anthocyanins*; optimal extraction; bio-waste valorization.

## INTRODUCTION

Aronia berries originate from the eastern North America, they are a perennial shrub within the *Rosaceae* family that is indigenous to that region of the world. There are three natural types of Aronia, *Aronia arbutifolia* (Red chokeberry), *Aronia prunifolia* (Purple chokeberry) and *aronia melanocarpa* (black chokeberry), with the latter being the most commonly specie cultivated [1].

Although the Aronia berries are native of North America, they were also cultivated in Europe and then diffused to Russia, Norway, and eastern Europe in the beginning of 20th century. In 1930, the Russian botanist Ivan Mitchurin, discovered the nutritious benefits Aronia, and its resistance to cold climates. The first cases of commercial cultivation of Aronia was registered in the 1980's in Sweden and Poland to produce natural red to replace artificial colorants in foods, juice, jams, wine and herbal tea [1]. Since then the Aronia cultivation increases mainly due to the scientific interest in antioxidants and their benefit for human health [2]. The interest area in Aronia, besides health, also includes the food industries interest in using natural colorants and food preservatives.

Anthocyanins are water-soluble red, blue and purple natural pigments responsible of the color of many fruits and plants such as Aronia [3]. The color of the berries given by anthocyanins serves as way to attract pollinators and seed dispersers and protect photosynthetic tissue from oxidative stress induced by light [4]. Anthocyanins are classified as polyphenols formed by phenylpropanoid metabolism from phenylalanine, and is synthesized by gymnosperms and most angiosperms.

Due to its antioxidative properties, anthocyanins are suited for fruit and vegetables preservatives, to both increase the shelf life and to protect against postharvest pathogens. An enrichment of anthocyanin in tomatoes was able to significantly extend their shelf life suppressing the ripening rate of tomatoes, and other crops [5].

In the optic of biomass valorization and avoiding the competition between food and compound recovery, aronia pomace from juice production was considered as potential source for anthocyanins recovery. This approach allows to valorize wastes that would end their life cycle in a landfill. Extraction temperature, solvent concentration and solid to solvent ratio were considered as fundamental variables to optimize the extraction process.

## MATERIALS AND METHODS

The extraction of anthocyanin was performed in a 1000 mL thermo jacketed batch reactor and each experiment was done in triplicates. The solvent considered for the extraction was citric acid monohydrate at the concentration of 0.25, 0,75, 1.5 wt %. The solvent was heated in the reactor at the experiment temperature before starting the extraction. Three temperatures were considered, 30, 50 and 70 ˚C.

After the temperature was stabilized, the aronia pomace was added. The pomace was provided frozen and was thawed overnight in a refrigerator at 5°C. Three amounts of pomace, 12.5, 20 or 50 grams were considered. They are equivalent to the following solid to solvent ratio, 1:80, 1:50 or 1:20. The batch extractions had a runtime of 30-60min, with samples taken at predefined times. Samples

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taken during the extraction process, were filtered through 0.2 $\mu m$ filters into HPLC vials.

Agilent Technologies 1200 HPLC was used to quantify the anthocyanin. The column used was a Gemini C18 5 $\mu m$ with the dimensions of 250 mm in length and with a 4.6 mm internal diameter, with a DAD. The mobile phases were water with 0.05 % TFA (A) and acetonitrile with 0.05 % TFA (B). with the following gradients, 0 to 1 min 99 % A, 1 to16 min 82 % A and 16 to 20 min 99 % A, with the injection volume of 20 $\mu l$ and a flow of 1 mL/min. The detection was carried out at 520 nm.

## RESULTS AND DISCUSSIONS

The influence of the different parameters on the extraction performance were reported in Figure 1.

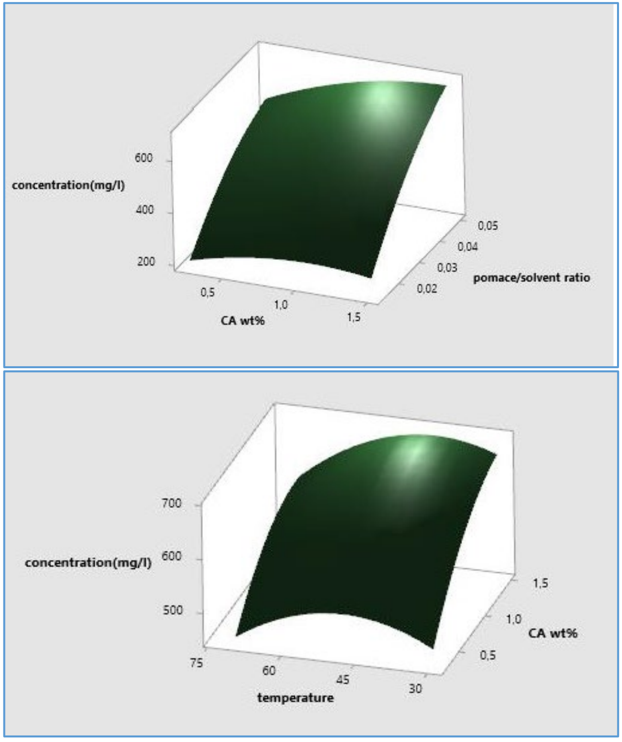

**Fig. 1:** Influence of citric acid (CA) concentration on anthocyanins concentration (up) temperature influence (down).

As expected, increasing the solvent concentration and the solid to solvent ratio the anthocyanins concentration increases. More interesting the same behavior was not observed for the temperature were a clear maximum in the concentration appears around 45 ˚C. Based on the analysis the maximum concentration was observed at 42 ˚C, a solvent concentration of 0.75 wt % and a solid to solvent ratio of 1:20 and it was equal to 691.77 mg/L.

## CONCLUSIONS

The objective of the study was to quantify and optimize the extraction of anthocyanin content from Aronia pomace. Three parameters were examined: temperature, pomace to solvent ratio and the concentration of citric acid in the solvent. All three parameters had a significant influence on the extraction of anthocyanins. The results showed an interesting potential for the valorization of this biowaste opening new potential markets for the Aronia juice producers. However, the optimization of the extraction step alone is not enough to assure the economical convenience of the production. The solvent removal after the extraction is the next challenge to overcome to bring the components to the market.

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# Red grape skin and seeds: extraction and characterization of flavonoids (anthocyanins) and wine production waste biomass valorization

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The last few years the interest in the exploitation of the winery residues is rapidly increasing. Both grape skin and seed extracts could become a valuable source of antioxidant compounds. Their valorisation towards that direction facilitates maintaining the environmental equilibrium. In the present study the method of producing an ethanolic extract of the red grape berries is presented and characteristics of it are shown.The method of the extraction process involved maceration followed by percolation; in the end of the process a strict protocol for quality control was applied. The extracts obtained via the specific extraction can be used either as individual medicinal for cures or in combination with other natural extracts to reestablish the healthy natural functions of the body. The current study, aims to production of pharmaceutical products such as capsules, tablets, drops, powder and elixirs from the red grape berries. The most important feature of this production is that all materials and products are non-toxic, natural and friendly to the environment.

**Key words:** red grape, extraction, flavonoids, anthocyanins.

## INTRODUCTION

From its humble origins, grape production has developed into the world's most important fresh fruit crop. Worldwide grape production in 2002 was about 62 million metric tons [1]. Approximately 66% of the production was fermented into wine [OIV, 2005]. Grape production is largely restricted to climate regions, similar to those of the indigenous range of *Vitis vinifera*. Grape culture is further largely restricted to regions characterized by Mediterranean-type climates [1].

The phenolic compounds in grapes (seed, skin, and pulp) can be divided into two groups, phenolic acids and flavonoids. Some flavonoids include catechin, epicatechin, procyanidins and their polymers and ester forms, quercetin, and red and blue anthocyanins. Flavonoids and related polyphenols from grape and grape seeds have generated remarkable interest based on positive reports of their antioxidant properties and ability to serve as free radical scavengers [2].

Flavonoids are natural products that are formed only in the bark or in the pulp of the fruit. The predominant flavonoids found in red grapes are anthocyanins and flavonols, which are almost exclusively present in glycosylated forms. They have antioxidant, anti-inflammatory, anti-allergic, diuretic, antibiotic and anti-carcinogenic properties. In particular, anthocyanins extracted from grape skins have been used as natural food colorants for over a century, but the compounds have recently received much attention due to their antioxidant activities and health promoting effects.

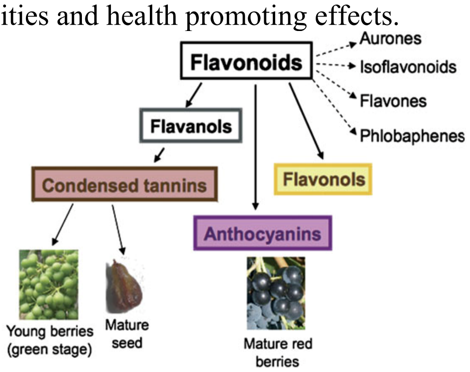

**Fig. 1.** The biosynthetic way of phenolic compounds leading to flavonoids, lignins and the main subgroups. [3]

Grape seed extracts are a major source of anthocyanins. Anthocyanins are of particular interest to the food colorant industry due to their ability to impart vibrant colors. They have been used as traditional herbal medicines due to their diverse physiological abilities to treat conditions such as hypertension, pyrexia, liver disorders, dysentery and diarrhea, urinary problems and the common cold. The consumption of anthocyanins may play a significant role in preventing lifestyle-related diseases such as cancer, diabetes, and cardiovascular and neurological diseases [4].

Red grape seeds appear to have the highest percentage (62% w/w) of phenolic compounds (catechins, pro-anthocyanidins, concentrated tannins), while red grape bark contains significant,

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but lees (33 % w/w) amount of phenolic compounds (anthocyanins, phenolic acids, flavonols, hydroxycinnamates).

Red grape skin and seeds have been used in order to produce a variety of pharmaceutical products [1].

In this work the medicinal properties of red grape skin and seeds (dried red grape berries) are presented; a method of valuable compounds extraction is shown; extracts characterization is demonstrated.

## MATERIALS AND METHODS

### *Solvents.*

Pure alcohol and deionized water were used as solvents.

### *Plant materials.*

Red Grapes were obtained from Greece. They were grown on the most suitable soil; only natural fertilization was employed; throughout their production synthetic fertilizers, pesticides, insecticides, fungicides or herbicides were not used.

### *Plant collection.*

The plant harvest was based on a strict protocol and collection rules. The harvest took place during the summer months (July-August) late in the morning to avoid moisture. After harvesting, meticulous cleaning of the red grape berries from all kinds of impurities (organic or inorganic) was done. Growth of harmful enzymes and fungi should be ruled out. Moisture is a key factor facilitating their growth. Therefore possibility of growth increases when the red grapes berries are packaged in liquid form. Thus this type of packaging was avoided. The transport length of the berries was as short as possible and it never exceeded 48 hours; the grape berries were transported in well-ventilated packages (perforated bags, baskets of reeds, boxes full of holes etc.).

### *Drying process.*

The red grapes berries first went through a process called stabilization, via which fungi and all bacteria that can cause berries decay were naturally eliminated. The method is as follows: the grape berries were positioned in a monolayer form on top of a sieve tray, where they were sprayed by a solution of 60 % pure ethanol, dispensed in a form of very fine droplets, so that a very thin layer of ethanol wetted all the external area of the berries. Ethanol was then left to evaporate.

The grape berries were dried. The drainage took place outdoors (sun or shade, it deepens) for 3 weeks.

### *Extraction method.*

A. MACERATION

The dried grape berries were crushed in the form of a coarse powder (5 mm). The powder was mixed with the solvent, which can be water, pure alcohol or a mixture of pure alcohol and water in proportion 65:35. The crushed seeds were allowed to soak for 3 days. During this process the mixture was shaken regularly and at the end of the third day the extract had a pulp like texture. This process is called maceration.

B. PERCOLATION

Percolation was the subsequently selected method of extraction. The dried grape berries pulp formed via maceration was placed in a special apparatus for 4 days with sufficient amount of pure ethanol, enough to completely cover the macerated mixture. It was then placed inside the percolator, a gravity driven form of extractor, of a specific diameter designed to process a well-defined mass of the macerated material according to the equipment specifications. After this process the extract was kept for 24 hours until the supernatant liquid was clear. Finally the liquid extract was separated via gravitational filtration.

### *Analysis.*

Quality controls were made during the procedure. In the end, the final quality control of the extract was conducted. Those control tests were based on strict protocols which involve analytical methods which have been developed in our laboratory through over 20 years of experience; they were based on traditional analytical methods of Galenea and French pharmacology but PANAX has advanced and specialized their applicability and reliability. It included determination of alcoholic degree and degree of acidity (pH) of the crude, fiber content, coefficient of expansion, bitterness limit and immediate anthocyanin identification which were used to confirm that the employed plant was red grape.

### *HPLC analysis.*

The samples were also analyzed using a Luna, 4.6×150 mm, 5 m particle size, 100 °A pore size column. A 40:60 acetonitrile:water solution was used as an isocratic mobile phase for 15 min at 0,5 ml/min flow rate and 30 ◦C. The injection volume was 20 microliter and detection was through a UV detector set at 235 nm.

## RESULTS AND DISCUSSION

The pharmacological and nutraceutical benefits derived from phenolic compounds are closely related to their antioxidant and singlet oxygen quenching ability. These phenolic compounds are able to trap and quench free radicals, and their antioxidant potentials have been shown to be four to five times that of vitamin C or E [2].

The results from the HPLC analysis of a tincture 10% dried grape berries extract are observed at figure 2. It can been seen that the extract contains a number of compounds manifested by the obvious overlapping peaks, but two of those predominate one at 2,950 min and one at 5,150 min.

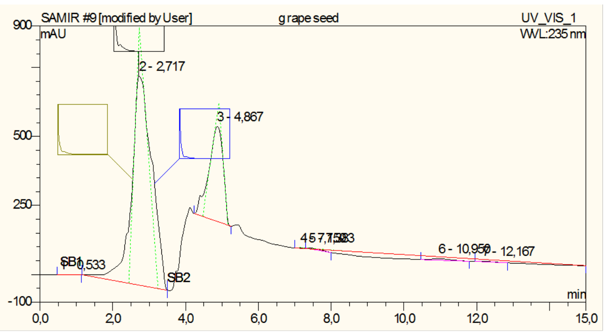

**Fig. 2.** HPLC absorption diagram at 235 nm, obtained from ethanolic dried grape berries extract. Absica shows retention time in min and ordinate arbitrary absorption units.

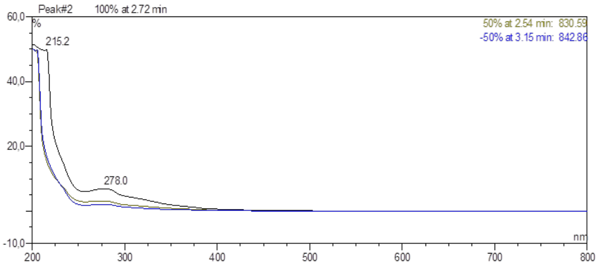

**Fig. 3**. Typical UV spectrum of the compound with retention time of 2.72 min of an extract from dried grape berries.

Figure 3 shows a typical UV spectrum of the peak at 2.72 min. As can be seen in Figure 3 the maximum UV absorption occurs at 215.2 and 278 nm. Further analysis for composition identification is currently underway.

In this work the treatment of dried grape berries to form ethanolic extracts. They lead to production of pharmaceutical products such as capsules, tablets, drops, powder and elixirs. The most important feature of this production is that all materials and products are non-toxic, natural and friendly to the environment.

## CONCLUSION

During the wine-making processes, a lot of by-products are produced, including grape skin and seeds. Grape skin, which represents about (5–10) % of the total dry weight of the grape berry, generally contains the highest concentration of tannin [5]. In this work we presented how an ethanolic extract of red grape berries was obtained; a preliminary characterization of it via HPLC and UV is shown. The obtained extract characteristics were tested by a number of methods verifying the quality of its identity.

***Acknowledgements:*** *This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 778168*

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# Butyric acid production by fermentation of waste hydrolysates

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Butyric acid is a valuable chemical with wide use in food and pharmaceutical industries. It is a precursor of butanol in acetone-butanol-ethanol fermentation (ABE). Usually, it is produced by different *Clostridia* strains from glucose as substrate. The aim of this study was to performed ABE fermentation by newly isolated *Clostridium beijerinckii* 4A1 strain, where glucose was replaced with hydrolysates obtained from several waste materials (distillers spent grains (DDGS), wood cellulose (WH) and spent coffee grounds (SCG)).The hydrolysates from spent grains and coffee grounds were prepared by acid hydrolysis followed by enzymatic hydrolysis. The wood cellulose hydrolysate was used as received from a local manufacturer. The direct use of waste biomass hydrolysates for butyric acid production was unsuccessful due to the toxicity of some products of the hydrolysis. For purification and detoxification, the hydrolysates were consecutively treated with Carrez solutions ($K_4[Fe(CN)_6]$, and $ZnSO_4$), $Ca(OH)_2$ and activated carbon. The waste biomass hydrolysates can be used after detoxification as substrates in ABE fermentation. Further optimization of the purification and detoxification as well as of the process parameter is necessary.

**Keywords:** Butyric acid, *Clostridium beijerinckii*, acetone-butanol-ethanol (ABE) fermentation, waste biomass hydrolysates, hydrolysates' purification.

## INTRODUCTION

Butyric acid (butanoic acid), a 4-carbon short-chain fatty acid, is widely used in chemical, food, and pharmaceutical industries.

Currently, butyric acid is predominantly produced from petrochemical feedstocks via chemical synthesis. Its production from renewable, low-cost biomass has attracted large attention in recent years.

In 1861, Louis Pasteur discovered that some rod-shaped microbes grew and produced butyric acid in the absence of air. The butyric-acid fermenting bacteria were divided into two groups: those producing mostly butyric acid as final product and those producing mostly butanol as final product. The latter process—called acetone–butanol–ethanol (ABE) fermentation—was one of the oldest known industrial fermentations. [1].

Many anaerobic microorganisms can produce butyric acid from sugars and other carbon sources. Generally, they pertain to the genera of *Clostridium, Butyrivibrio, and Butyribacterium* [2].

Usually, the optimal conditions for butyric acid fermentation are 35–37°C in the anerobic atmosphere (pure $CO_2$, $N_2$ or a 1:9 mixture of $N_2$ and $CO_2$ [3]) and a pH range of 4.5–7.0. The pH value depends on the objective of the bioprocess because the pH optima for acidogenesis and solventogenesis differ [4].

Glucose is the common carbon source for butyrate or butanol production with *Clostridium*q but *Clostridium* bacteria are able to utilize a wide range of sugars: hexoses, several pentoses and oligo- and polysaccharides. Lactose from whey [2, 5, 6], saccharose from molasses [7], starch [8], potato wastes [9], wheat flour [10], cellulose [11] are among the possible substrates. For industrial application, a non-pathogenic strain is preferred for environmental health and safety concerns.

Slow cell growth has a positive effect on butyrate productivity and selectivity. It may be evoked by carbon limitation in continuous or fed-batch processes. Higher butyrate concentrations may be obtained in fed-batch cultures than in continuous cultures. On the other hand, higher productivity may be achieved through the use of continuous cultures [12, 13]. Strains screen, substrates selection and innovation in fermentation techniques are in the research focus in order to make ABE fermentation engineering sustainable and economically feasible.

One of the ways to achieve cost-effective and industrial-scale fermentation of butyric acid is to use waste biomass as fermentation substrate. Lignocellulosic biomass is considered as the most abundant and inexpensive biomass on the earth [14] and it is a rich, sustainable carbon source which uses as a substrate helps in avoiding the global food crisis [15].

However, as a result of the pretreatment and hydrolysis of lignocellulose by diluted acids, several kinds of microbial inhibitors such as phenolic compounds, furan derivatives, some organic acids, etc. [6], which have been confirmed to seriously suppress cell growth and product synthesis [16].

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Although detoxification step has been proved to effectively reduce the toxicity of lignocellulosic hydrolysates, it may significantly increase the total costs of biofuels and bio-based chemicals due to the separation process step and sugar loss [17].

In this study, hydrolysates, obtained from different waste materials (distillers spent grains (DDGS), wood cellulose (WH) and spent coffee grounds (SCG)) were used as substrates for butyric acid production by newly isolated *Clostridium beijerinckii 4A1* strain.

## MATERIALS AND METHODS

### *Raw materials*

Spent coffee grounds were taken from a local cafeteria, DDGS a by-product of the ethanol production from grain was received from Almagest AG, Ihtiman, Bulgaria. The wood hydrolysate was obtained from local producer.

### *Strain and medium*

In this work, a newly isolated *Clostridium beijerinckii* 4A1 strain from chickpea fermentation was used [18]. Reinforced Clostridial Medium (RCM) was used as a basic medium for strain maintenance. The inoculum was prepared by growing of 1 ml the culture in 10 ml sterile culture medium and incubated for 24 hours at 37 ºC at anaerobic conditions achieved by Anaerocult® A (Merck Millipore, Germany). The thus prepared inoculum was used in all subsequent experiments. The experiments were performed on hydrolysates, replacing glucose in RCM.

### *Hydrolysates preparation and purification*

The hydrolysates from spent grains and coffee grounds were prepared as follows: 500 ml stock solution (10% w/v dry material) was hydrolyzed in an autoclave with 1% $H_2SO_4$ at 2 atm for 1 h. The resulting hydrolysate was treated with cellulase „Onozuka R-10" (Yakult Pharmaceutical Industries Co., Ltd. Japan) for 24h at pH=4.5 and 45 ºC (20 U/g substrate). Aliquots were taken for fermentation with appropriate reducing sugar concentration. The wood cellulose hydrolysate was used as received from a local manufacturer.

For purification and detoxification, the hydrolysates were treated with Carrez solutions ($K_4[Fe(CN)_6]$, and $ZnSO_4$) for protein precipitation. After filtration of the precipitated protein, $Ca(OH)_2$ was added to pH about 10 for partial removal of inhibitors and elimination of the excess of sulfate ions. The pH of the filtered solution was corrected with $H_2SO_4$ to 5.5 and $Na_2SO_3$ (0.1%) was added. The solution was boiled for 3 h. The initial volume of the solution was restored with distilled water, and activated carbon (10% w/v) was added for as complete as possible removal of furans, acids and other inhibitors. After shaking for 1 hour at room temperature the filtered hydrolysates were used for fermentation media preparation.

### *Analysis*

The concentration of the target products (sugars, butyric acid, and butanol) was determined by HPLC. An Aminex HPX- 87H, 300x7,8 mm column and 0,01 N $H_2SO_4$ as mobile phase at a flow rate of 0.6 ml/min were used. The biomass concentration was determined by optical density measurements at 620 nm with a spectrophotometer VWR UV-1600PC.

## RESULTS AND DISCUSSION

The maximum theoretical yield of butyric acid determined from the following stoichiometric equations:

$$C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2H_2 + 2CO_2 \quad (1)$$

$$6C_5H_{10}O_5 \rightarrow 5C_4H_8O_2 + 10H_2 + 10CO_2 \quad (2)$$

is 0.49 g/g from hexoses and 0.59 g/g from pentoses. In case of a cellulosic hydrolysate with undefined sugar ratio, the yield should be between these values. However, in a real fermentation, the yield would be lower due to production of acetic and lactic acids.

As it was mentioned above, during acid pretreatment of cellulosic substrates, together with individual sugars and oligosaccharides different organic compounds are also released - furan derivatives, phenolic compounds, and weak acids. These compounds are usually strong microbial inhibitors. For example, the furan derivatives in hydrolysates are derived from the degradation of monosaccharides. They are identified as notorious fermentation inhibitors, which have been confirmed to seriously suppress cell growth and product synthesis by disrupting cell membranes and nucleic acids, inhibiting the activity of key enzymes, and causing intracellular oxidative stress response [18]. Hence, when the lignocellulosic biomass was hydrolyzed by dilute acid, which is an efficient, economical and widely used method, the furan derivatives would become major inhibitors and severely inhibit cell growth.

Table 1 shows the concentration of reducing sugars in hydrolysates at a different stage of pretreatment. The purification led to a loss of sugars from 5 to 30%.

Usually, a typical ABE fermentation starts with about 60 g/l glucose or other substrate and continues from 32 to 72 h. Preliminary studies on the influence of glucose concentration showed that the optimal one for the strain *Clostridium beijerinckii* 4A1 is 20 g/l (Table 2).

**Table 1.** Reducing sugars concentrations of different substrates

| Substrate | Reducing sugars, g/l | | |
|---|---|---|---|
| | After acid hydrolysis | After enzyme hydrolysis | After purification |
| DDGS | 19 | 24 | 23 |
| SCG | 21 | 26 | 18 |
| WH | 10 | 10 | 7 |

**Table 2.** Influence of the glucose concentration on the growth and production of *Clostridium beijerinckii* 4A1

| Glucose g/l | Biomass g/l | Butyric acid g/l | C/N ratio |
|---|---|---|---|
| 5 | 4.97 | 1.25 | 0.734 |
| 10 | 7.43 | 3.67 | 1.468 |
| 15 | 10,41 | 4.95 | 2.202 |
| 20 | 13,00 | 5.47 | 2.936 |
| 50 | 6,52 | 2.45 | 7.339 |

The results of a typical ABE fermentation by *Clostridium beijerinckii* 4A1 are presented in Figure 1.

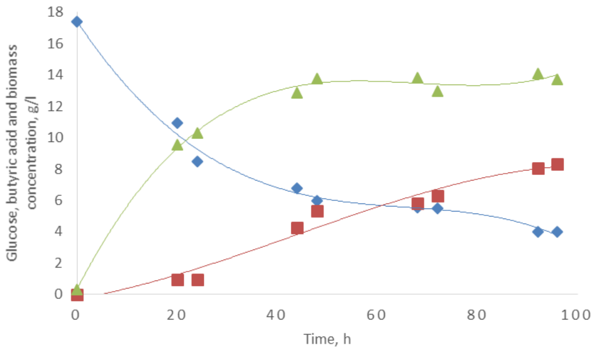

**Fig. 1.** Time course of glucose (◆), butyric acid (■) and biomass (▲). Initial glucose concentration ~20 g/l.

The first set of experiments for microbial butyric acid production were carried out with obtained hydrolysates (after acid and enzyme hydrolysis) added directly to the medium without purification. The initial reducing sugars concentration was 10 g/l.

The results of the experiments with non-treated hydrolysates are presented in Fig. 2.

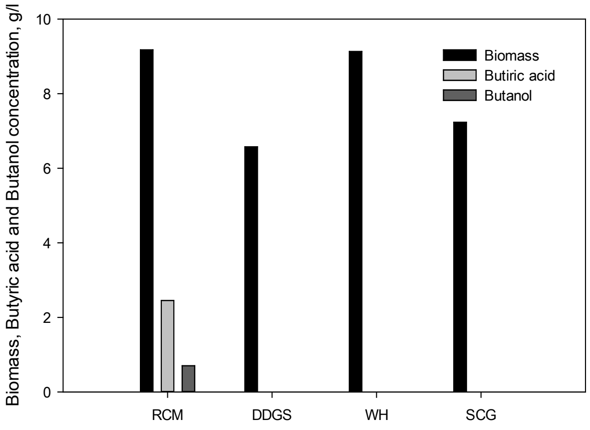

**Fig. 2.** Biomass growth and butyric acid and butanol production on untreated hydrolysates.

As can be seen from the figure, despite the good biomass growth neither butyric acid nor butanol were produced.

Baroi *et al.* [19] reported that *Clostridium tyrobutyricum* strain does not grow in pretreated by wet explosion and enzyme hydrolysis wheat straw hydrolysate.

Although detoxification step has been proved to effectively reduce the toxicity of lignocellulosic hydrolysate, it may significantly increase the total costs of biofuels and bio-based chemicals due to the separate process step and sugar loss [20].

After hydrolysates' purification and detoxification, according to the above described three steps scheme a new set of experiments was carried out. An RCM medium, containing about 7 g/l reducing sugars (this was the sugar's concentration in wood hydrolysate after detoxification treatment) was used as a control.

Purification of the hydrolysates improved the butyric acid production to 68-78% from the control. The results are shown in Fig. 3.

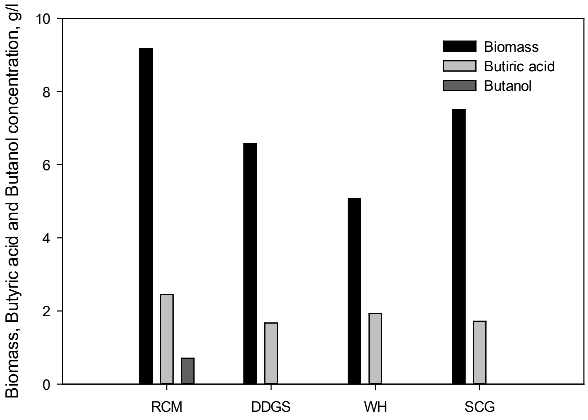

**Fig. 3.** Biomass growth and butyric acid and butanol production on treated hydrolysates.

Zhang *et al.* [21] reported differences in the degree of detoxification using different methods. While overliming removed more dialdehydes and diketones, treatment with active carbon (AC) removed more phenolic acids. The authors suggested that a combination of different detoxification methods was needed for ABE fermentation. The sequential overliming and AC treatment resulted in remarkable fermentability and high butanol yield (88% from the control with glucose as substrate).

## CONCLUSIONS

The direct use of waste biomass hydrolysates for butyric acid production was unsuccessful due to the severe toxicity of some products of the hydrolysis. The waste biomass hydrolysates can be used, after detoxification, as substrates in ABE fermentation with good productivity (68-78 % from the control).

Further optimization of the purification and detoxification, as well as, of the process parameter is necessary.

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# Towards improved valorisation of (*Betula pendula)*: preliminary study of the genotoxic, antigenotoxic and cytotoxic potential of a commercial aqueous silver birch leaf extract

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Birch is a tree, of the genus *Betula* belonging to the *Betulaceae* family and it is known for its various pharmaceutical properties. At the same time it is a suitable tree species for renewable forests. In the present preliminary study an aqueous commercially available leaf extract of silver birch (*Betula pendula)* was studied to evaluate its potential genotoxic and cytotoxic activity as well as its antigenotoxic properties against the mutagenic agent *mitomycin-C* by employing the *in vitro* Cytokinesis Block Micro Nucleus (CBMN) assay. Human lymphocytes were treated with *Betula pendula* with and without *mitomycin-C*. *Betula pendula* did not increase the frequency of micronuclei, and showed cytotoxic potential. All mixtures of *Betula pendula* and *mitomycin-C* demonstrated a decrease in the micronuclei frequencies, with the lowest and highest concentrations inducing a significant antigenotoxic activity. Therefore, the birch product studied showed antigenotoxic and cytotoxic potential which could render it useful in various medicinal applications.

**Key words:** natural medicines, anticancer properties, silver birch leaf-extracts, CBMN assay

## INTRODUCTION

Silver birch (*Betula pendula* Roth.) is one of the most important forest-forming and timber production trees. In Poland in particular, approximately 2.5 million cubic metres of birch wood timber is sourced annually by the State Forests. Birch wood is being used for paper and furniture production, as firewood and for certain pharmaceutical, cosmetics and food products. However, all parts of the silver birch can form valuable biomass for biofuel production. Towards that direction its composition has been characterised by Lachowicz *et al.* [1]. Uri *et al.* [2] have studies the carbon sequestration ability of the same species, which is important to know when trees are to be used for biofuel production. However, biomass is most valuable when it is first used as pharmaceuticals or natural additives, with food and animal feed to follow, chemicals production to form the third option and biofuels production as the ultimate between the aforementioned one.

Birch is a tree source of natural pharmaceutical products. As such, the valorisation of birch biomass should start from the extraction of valuable products. Since ancient times natural products have found multiple applications in various fields and demonstrated remarkable pharmacological and medicinal properties. A great deal of the knowledge of those properties is lost; additionally such knowledge was frequently based on empirical data and the science underpinning their beneficial properties has to be established. Their thorough study could lead to the discovery of more beneficial properties and their establishment as promising agents against several diseases. This work focuses on such properties of the birch, to demonstrate the added value products which can be obtained from such biomass before its exploitation for energy generation.

Birch is a tree, of the genus *Betula* belonging to the *Betulaceae* family and is most common in the northern hemisphere. It is known for its pharmaceutical properties while many studies have been conducted indicating that the plants themselves as well as components from various birch species possess antimicrobial, antioxidant and anticancer activity (Rastogi *et al*. [3]).However, most research papers have focused on the chemical composition and various properties of the birch bark and its constituents (Zhanataev *et al.* [4], Sami *et al*, [5], Calliste *et al*, [6]). There is very limited scientific work on the properties of the birch leaves.

In this research, the possible genotoxic and cytotoxic activity of an aqueous leaf extract of birch (*Betula pendula)*, as well as its antigenotoxic potential was studied in cultured human lymphocytes applying the cytokinesis block micronucleus (CBMN) assay for the detection of micronuclei in the cytoplasm of interphase human lymphocytes. Micronuclei may originate from acentric chromosome fragments or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. Thus, this assay detects the potential clastogenic and

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aneugenicactivity of chemicals in cells that have undergone cell division after exposure to the test chemical (OECD, [7], Kirsch-Volders *et al.* [8]).The antigenotoxic effect of the birch product was studied against the genotoxic damage induced by mitomycin C (MMC), an antitumor compound that has a range of genotoxic effects including the inhibition of DNA synthesis, mutagenesis and clastogenesis.

## MATERIALS AND METHODS

### *Chemicals*

The aqueous leaf extract of *Betula pendula* was purchased by Abnoba GmbH (http://www.abnoba.de/, Pforzheim, Germany; Betula folium D3 Abnoba, batch-no. 706 A41 was used for the investigations). Mitomycin C and Cytochalasin-B (Cyt-B) were purchased from Sigma (St. Louis, MO, USA). Ham's F-10 medium, foetal bovine serum and phytohaemaglutinin were commercially supplied (Gibco, UK). All other chemicals and solvents were of the highest grade commercially available. Stocks of the compounds and solutions were stored at 4°C until use.

### *Ethics Statement*

The study was approved by the Ethical Committee of the University of Patras. After informed consent healthy, non-smoking male individuals (less than 30 years), were used as blood donors to establish whole blood lymphocyte cultures. According to the donors' declaration, they were not exposed to radiation, drug treatment or any viral infection in the recent past.

### *CBMN assay in human lymphocytes in vitro*

The CBMN assay was performed according to standard procedures (OECD 487 [7]) with minor modifications. Human blood samples were obtained from two non-smoking, healthy individuals not undergoing any drug treatment, having viral infection, or having X-ray exposure in the recent past. Whole blood (0.5 mL) was added to 6.5 mL of Ham's F-10 medium containing 1.5 mL of fetal bovine serum and 0.3 mL of phytohaemagglutinin to stimulate cell division. The birch product was studied at four different doses i.e. 0.5, 1, 2 and 5% (v/v) of the total culture volume and *Mitomycin C*(0.05 μg/ml) was given along with each of the reported doses of the birch-leaf product, 24 h after culture initiation.

After 44 h of incubation, cytochalasin-B (final concentration of 6 μg/ml) was added to the cultures to block cytokinesis of dividing cells. Cultures were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ for 72 h; 72 h after the initiation of culture, cells were harvested and collected by centrifugation. A mild hypotonic treatment with 3:1 solution of Ham's medium and milli-q $H_2O$ was left for 3 min at room temperature which was followed by 10 min fixation (for at least 3 times) with a fresh 5:1 solution of methanol/acetic acid. Cells were stained for 10 min with 7% Giemsa (Vlastos *et al.* [10]). Binucleated cells with preserved cytoplasm were scored per experimental point to calculate the micronuclei frequency according to standard criteria (Fenech [11] and Fenech *et al.* [12]).

To determine possible cytotoxic effects, the cytokinesis block proliferation index (CBPI) was evaluated (Surrallés *et al.* [13]). CBPI is given by the equation:

$$CBPI = \frac{\mathrm{M1} + 2\mathrm{M2} + 3(\mathrm{M3} + \mathrm{M4})}{\mathrm{N}} \qquad (1)$$

where M1, M2, M3 and M4 correspond to the number of cells with one, two, three and four nuclei, respectively and N is the total number of counted cells.

## RESULTS

As mentioned above, the birch-leaf product was studied at four different doses i.e. 0.5, 1, 2 and 5% (v/v) of the total culture volume and the same doses were tested combined with *Mitomycin C* at a concentration of 0.05 μg/ml for *Betula pendula* in order to identify its antigenotoxic effect against the genotoxic damage induced by *Mitomycin C*. Treatment with the *Betula pendula* did not induce micronuclei as compared to control. Treatment with 0.05 μg/ml of *Mitomycin C* induced an increase in micronuclei frequencies, as expected, compared to control. A decrease in micronuclei frequency was found when the lowest and highest concentration of *Betula pendula* was given along with the concentration of *Mitomycin C*.

The cytotoxic effect of the *Betula pendula* and its mixture with *Mitomycin C* was evaluated by the determination of CBPI. Regarding the cytotoxic index, significant differences on CBPI were detected between control cultures and the two highest doses of the *Betula pendula*. Moreover, significant cytotoxic effect appeared between *Mitomycin C* and one of the *Betula pendula* concentrations with *Mitomycin C*.

## DISCUSSION

Natural products have been used since antiquity and are known for possessing remarkable medicinal and pharmaceutical properties. In the present study the genotoxic and cytotoxic effects of a birch (*Betula pendula*) aqueous extract (*Betula pendula*) was screened to evaluate its antigenotoxic potential. For this purpose the *in vitro* Cytokinesis Block MicroNucleus (CBMN) assay was employed.

The lack of genotoxicity showed by the *Betula pendula* could be explained by its major phenolic components which were found to be quercetin-monoglycosides by the phytochemical analysis of the specific product conducted in a previous study (Gründemann *et al*, [14]). Quercetin's genotoxic potential has been evaluated in various studies and it has been concluded that this naturally occurring flavonol is not considered genotoxic (Da Silva *et al*. [15]; Utesch *et al*. [16]).

The potential antigenotoxic activity of the *Betula pendula* was examined by co-treatment of human lymphocytes with the *Betula pendula* and the mutagenic inducer *Mitomycin C*. *Mitomycin C* is an antibiotic that transforms into an alkylating agent and affects DNA synthesis, causes inter-strand cross-links in DNA and formation of DNA adducts (Iyer and Szybalski [17], Waring [18], Dall'Acqua *et al* [19], Bargonetti *et al* [20]). It has been found to be genotoxic in all *in vitro* and *in vivo* test systems in mammalian cells and animals, thusbeing considered as carcinogenic agent (Lorge *et al*, [21]; Mazumdar *et al*, [22]). Accordingly, *Mitomycin C* was found to be genotoxic, inducing statistically significant increase in micronuclei and BNMN.It was observed that all concentrations of the *Betula pendula* led to a decrease in micronuclei frequency which was statistically significant in the lowest and highest concentrations tested. The induction of antigenotoxic potential could be attributed to the antigenotoxic activity of *Betula pendula*'s most abundant flavonol, quercetin. Specifically, quercetin prevented DNA damage and had antiproliferative properties in human hepatoma cell line (HepG2) against *tert*-butyl hydroperoxide (*t*-BHP), in addition to increasing the rate of DNA repair (Ramos *et al* [23]). Moreover, quercetin exhibited antigenotoxic effect *in vivo* against chromium trioxide induced micronuclei in polychromatic erythrocytes of mouse peripheral blood (García-Rodriguez *et al*. [24]).

As far as the cytotoxicity of the *Betula pendula* is concerned, a significant decrease of CBPI values was observed at the two highest concentrations of the *Betula pendula*, as well as at one concentration of the *Betula pendula* with *Mitomycin C*. The induction of cytotoxicity by the *Betula pendula* can be corroborated by past studies concerning various Betula species. In a previous study by Goun *et al* [25], the methylene chloride and the methanol extracts of *Betula pendula* were evaluated for their activity against leukemia. A cytotoxicity assay, determining the inhibitory effect of test samples on the growth of mouse leukemia cells (L1210), was applied, with the extracts exhibiting a high level of cytotoxicity against leukemia. *Betula pendula* fractions were tested and showed antiproliferative activity against B16 melanoma cells (Calliste *et al* [6]). Another Betula species, *Betula platyphylla* var. *japonica* was tested and its extract induced apoptotic cell death in human promyelocytic leukemia (HL-60) cells, a cancer cell line (Ju *et al* [26]).

Since most natural substances constitute a mixture of various components, their potential beneficial effects could be attributed to the synergism among the different components (Liu RH, [27], Koutsoudaki *et al*, [28] Vlastos *et al* [29]). In addition to that, the mixture of different substances or natural products could also lead to enhanced effects (Huh *et al* [30]).

## CONCLUSION

In conclusion, our preliminary study provides evidence on the antigenotoxic potential, induction of cytotoxicity and the lack of genotoxic activity of a birch product under the present experimental conditions. Further research could be suggested to identify potential beneficial properties of birch. As such, the production of added value product from birch, before it is used for energy generation is possible and should be examined. This study was employing a commercially available leaf extract. However, the conditions of production may have an effect on the qualities of the final product and as such, its properties will be examined under a variety of extraction methods.

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# On the way to circular economy: a systems approach towards sustainable *Arctium Lappa* valorization via valuable medicinal products extraction

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In our work for the first time is outlined the application of a novel methodology to examine whether the roots of *Arctium Lappa*, commonly known as burdock, can be considered a potential feedstock for a biorefinery targeted to producing valuable medicinal products with a wide spectrum of applications. The biorefinery should implement predominantly mild, advanced green processes and techniques that will allow obtaining from the burdock roots the high value added products without damaging one or more of the extracts, particularly those that are heat sensitive. The biorefinery sustainability will be determined using life cycle and techno-economic assessment analyses. The residual biomass will be available for the production of further products of the same or potentially lower added value than the primary extracts from the roots. Via further steps, the entire mass of the plant can be used so that zero waste is possible.

**Key words:** *Arctium lappa*, pharmacological activity, biorefineries, life cycle assessment

## INTRODUCTION

*Arctium lappa* is a traditional Chinese medicinal herb and an edible perennial plant of the *Asteraceae* family. Popularly known as burdock, this plant is native to Europe and Asia and was rapidly spread across North America by the early European settlers.

The different parts of the plant possess antioxidant [1-5], antibacterial [1, 5-7] and anti-inflammatory biological activities [5, 8-9] and are commonly used to treat various illnesses, such as throat infections, intoxications, skin infections and to relieve rheumatic pain and fever.

Recently, it was confirmed that *A. lappa* is capable of improving mucus protection in the stomach and intestines, besides preventing mucous injuries caused by alcohol [10]. Some burdock components have been reported to possess pharmacological activities like antioxidant, anti-inflammatory, antiproliferative and antiviral [11-15].

Burdock thus, can be a potential feedstock for a wide variety of chemicals. However, as an option for extraction of all of these components from the different parts of a single plant, the economic viability and environmental impacts for cultivation and conversion need to be studied for the plant species. This plant is known to originate from the northern parts of Europe and Asia, but also can be found around the world; because of its easy and rapid acclimatization. It is classified as an invasive weed in the majority of countries outside its native range, which makes it very suitable as a feedstock for the production of useful products as its utilisation will simultaneously form a method of control of its spread. At the same time, it develops to a rather large bush which allows the collection of substantial biomass from a single plant.

In our work for the first time, the application of a novel methodology for multi-objective optimization and systems integration to monetarizing burdock into added value products is sketched. It involves an outline of plant collection and the presentation of a simplified process for the production of added value products from the most valuable part of the plant, its roots. Added value products of this kind can greatly improve the viability of the further exploration of the respective biomass. Alternative/additional processes can be used for the extraction of added value products from other parts of the plant. The remaining biomass can be valorised towards the production of additional products. The valorisation of this biomass forms an unprecedented opportunity for the implementation and development of the-top-to-bottom, bottom-to-top coupled method, described in detail by Pham and El Halwagi [16]. The method can be also coupled with the atomic targeting and design as presented by El-Halwagi in [17]. Furthermore, the work of Sengupta and Pike [18] focusing on the

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production of different chemicals including biofuels from waste biomass provides the foundation for the further exploitation of this biomass and a methodology to follow towards that objective.

## METHODOLOGY

However, as an option for extraction of all of these components from the different parts of a single plant, the economic viability and environmental impacts for cultivation and conversion need to be studied. Figure 1 displays a macroscopic, overall procedure, which can form a starting point for the development of a network of options, the viability of which needs subsequently to be examined.

The selection of the feedstock is the first step. Feedstock can be collected either as a weed from abandoned uncultivated land or obtained as a cultivated product. In the former case, the cost of the land and its cultivation can be considered negligible; however, the control over the desired quantity and probably quality of the feedstock may be questionable and it is greatly condition depended. Cultivated on-purpose burdock is not subject to all of the above drawbacks. However, the costs of cultivation (including irrigation, fertilizing of land, other weed and insects controls, potential weather protection means) add to the costs of its production. Of course a combination of the above two sources of origin could potentially form a much better solution.

The collection of the feedstock is another important aspect. Burdock roots are the most important part of the plant. Thus, deep digging may be necessary rendering the accessibility of the land and the capacity to dig out the burdock plants - an issue of key importance. The cultivated crop fields can be designed in advance, in a way which makes crop collection easier, while the selection of the land for the cultivation can be such, so as to minimalise the transportation costs. Of course, freely grown burdock plants may also be accessible and Figure 1.

Feedstock selection, collection and pretreatment scheme be encountered in locations where transportation is easy.

Depending on the timeline and the procedures followed, the collection of the feedstock and the conditions of its transportation may impose that preservation and pretreatment of the feedstock is necessary, either as steps immediately following collection of the plants or as steps subsequent to their transportation activity.

The best time for the feedstock collection is in the summer, when most of the above ground sections of the plant are almost dry, but the roots are still containing a high amount of moisture. The plants can be dug out and left to dry outside, exploiting the solar energy and contributing to the viability of the process.

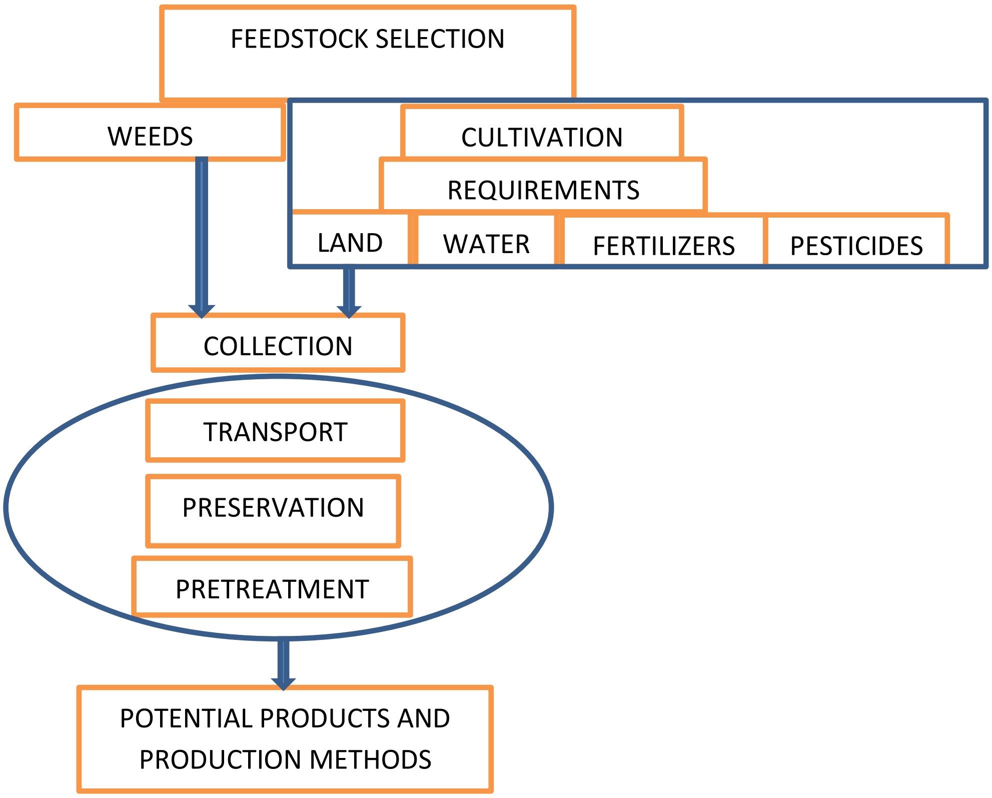

**Fig.1**: Feedstock selection, collection and pretreatment scheme

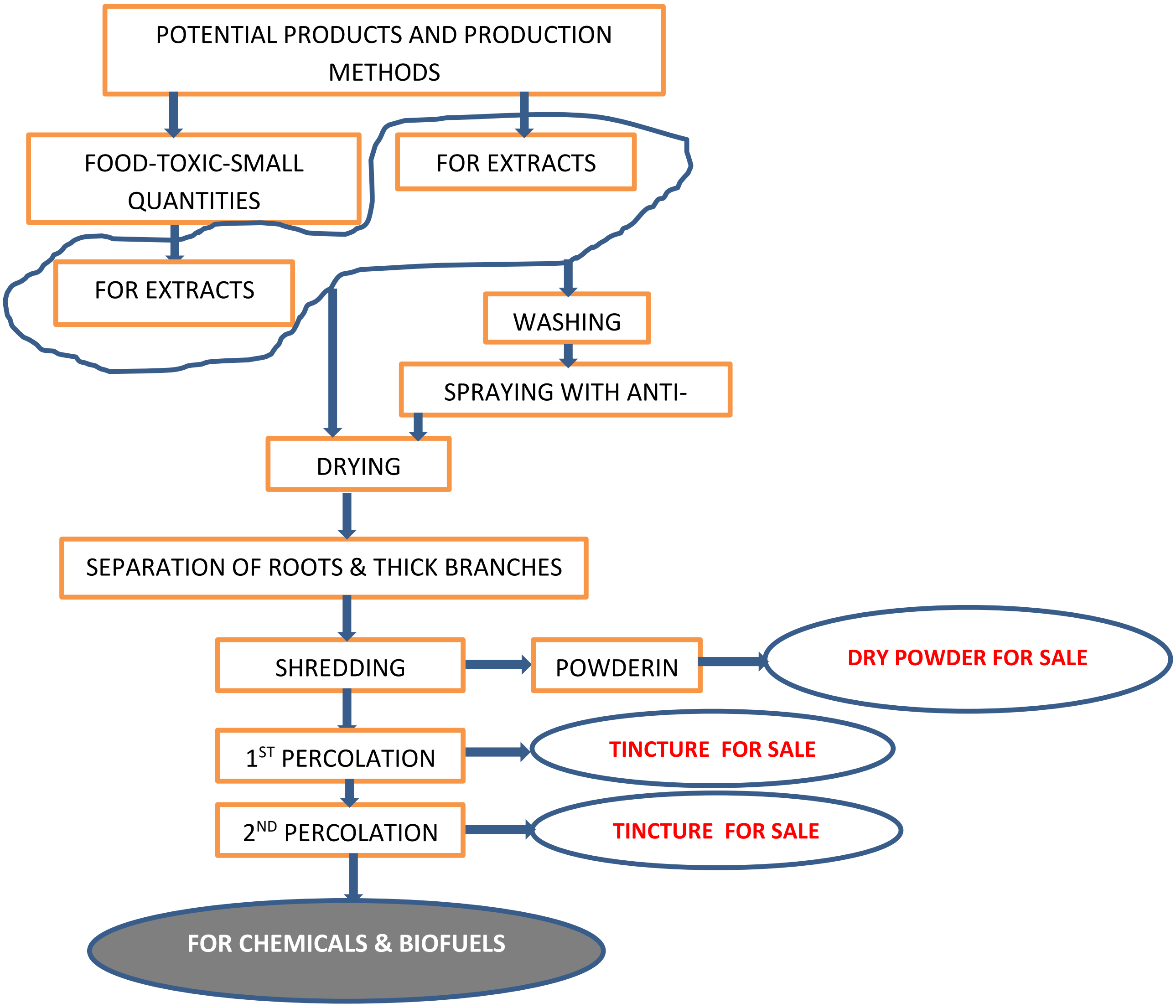

**Fig. 2:** Valuable natural medicines production steps

Moreover, mobile units can be employed for the treatment of parts of the plant, so that only part of it needs to be transported to a central unit for further treatment.

Figure 2 shows the necessary subsequent steps for the production of added value products.

The plants can be dried as collected, or washed first and then dried. In the latter case, spraying of the plants with a mold preventing solution is necessary. In the former, the need of such mold prevention pretreatment depends on the conditions *i.e.* the ambient moisture and whether a possibility of fungi growth on the plant exists. For high quality medicinal products and providing that the measures to prevent mold development are in place, the drying of the plants is better to be done in an open air-shaded place. The use of furnaces is not recommended.

The dried plants are then separated into leaves and roots; thick branches are collected with the roots. They are consequently shredded. The shredded parts can be either milled further to form a powder which can be sold or used for the preparation of formulations, or can be used for making tinctures *of arctium lappa*. The latter is done through percolation employing 60° ethanol in water solution. After removing the liquid extract, a second percolation can follow using a 1:10 by weight mixture of ethanol:wet solid. The tinctures and powders obtained via this procedure possess excellent, powerful, but at the same time gentle for the body medicinal properties. Being very valuable products can have a significant contribution towards the viability of an integrated process which subsequently valorizes the remaining biomass from this plant.

At present, burdock is underused and there are just a few studies in the literature devoted to the obtainment, characterization and application of its valuable extracts. Burdock root extractions with supercritical carbon dioxide ($scCO_2$) and compressed propane, characterization and biological activities of the extracts, influence of process conditions (pressure and temperature) and solvent effects on the chemical composition, total phenolic content and antioxidant activity were reported in [1,2].

In order to examine a multitude of alternative techniques to the same feedstock (burdock leaves), de Souza *et al.* [19] studied the capabilities of pressurized liquid extraction with hydroalcoholic solutions with different mass fractions of water, and compared those to the results obtained by: i) a sequential six step supercritical extraction with ethanol as a co-solvent and ii) a supercritical extraction procedure where distilled water was added to the ethanol as an auxiliary solvent, referred to as $scCO_2$+Aq. EtOH procedure. Furthermore, the PLE burdock leaves extracts contained higher concentrations of chlorogenic acid and rutin and exhibited considerably high DPPH free radical scavenging activity [19].

Major compounds found in the burdock root extracts were diisooctyl phthalate (DIOP) and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), glycerol, methyl oleate, butanoic acid and pentadecanal. Analogous experiments were performed on the areal parts (leaves) of the plant by de Souza *et al.* [2]. A number of important phenolic compounds like lupeol acetate, amyrin acetate, diisooctyl phthalate and phytol were identified in the extracts obtained and it was concluded that supercritical extraction with ethanol as a co-solvent has a potential as a viable technique.

The promise to replace fossil sources employing the remaining waste biomass of burdock as a feedstock allows a step towards circular economy, and expands the potential to establish an alternative supply chain which can be further studied in a wholesome mode through life cycle assessment.

The feasibility of a biorefinery including decisions on scale, processes, products, water use, and waste management through the entire plant, needs to be studied by means of a techno-economic assessment. Finally, the environmental impacts and the safety of the processes need to be quantified for the processes that convert burdock to products, including transportation of the species to the biorefinery.

Therefore, burdock can be a potential feedstock for a wide variety of chemicals. However, as an option for extraction of all of the aforementioned components from a single plant, the economic viability and environmental impacts for cultivation and conversion need to be studied for the plant species. The multiobjective framework will be applied, where potential tradeoffs are considered. The production of high added value products via the exploitation of its roots and thick stems as shown schematically in Figures 1 and 2 should be the first priority. However, for the production of high value pharmaceuticals, organic, good quality plants are an obvious necessity. Less qualitative plants together with the leftover biomass from the aforementioned extraction process can be used for the production of valuable chemicals. For that purpose, the valuable compounds identified to be contained in burdock biomass by previous research have to be quantified. The waste biomass after those processes can be further used for biofuel production via anaerobic digestion for instance, or to they can be further treated for the formation of adsorbents, compost, etc.

## CONCLUSION

The ultimate goal of this work is to study whether burdock can be considered as a potential feedstock and to determine the viability using life cycle assessment and techno-economic assessment analyses.

Using process integration, the traditional approaches can be modified if it is infeasible, or to identify product and production pathways which will improve its feasibility.

For that purpose, a systems-based “forward-backward” approach as outlined by Pham & El Halwagi [16] will be followed. The forward part aims at identifying the possible intermediates while the reverse synthesis starts with desired products and moves “backwards” for the identification of pathways leading to them. The optimization process will involve the formation of sub-problems. For each of them, the available technologies and the best policy will be examined. Subsequently, appropriate programming algorithms will be developed to determine the optimal pathways.

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# Avocado seeds valorization as adsorbents of priority pollutants from water

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This study has focused on the synthesis of lanthanum-, cerium- and calcium-functionalized adsorbents to remove heavy metal ions from aqueous solutions. Avocado seeds were used as a feedstock to obtain carbon-based adsorbents via pyrolysis and these adsorbents were further functionalized with lanthanum, cerium and calcium species using different experimental conditions to improve its adsorption properties. These adsorbents were utilized for the adsorption of heavy metals from aqueous solutions. At tested experimental conditions, the raw adsorbent showed an adsorption capacity of 7.04, 10.91, 11.41 and 6.38 mg/g for cadmium, nickel, copper and zinc, respectively. These adsorption capacities increased up to 70 % after adsorbent surface functionalization. These results suggested that the adsorption capacity of avocado-based adsorbents appeared to be dependent of the adsorbate properties.

**Key words:** Adsorption, heavy metals, lignocellulosic waste, water purification, avocado seed.

## INTRODUCTION

Agroindustrial wastes generated by agricultural, forestry and industrial activities including by-products of agrifood industry are generated in high amounts every year. Specifically, it has been estimated that around 1550 million of tons are generated annually [1] where 80 % of this biomass is burning (for heating, cooking, charcoal production, and the generation of steam for mechanical and electric power applications), 15 % is used as animal feed, 4.5 % is reincorporated to the ground without previous decomposition and the remaining 0.5 % is used as raw material in industrial processes [2,3]. These wastes are considered as renewable organic materials with high content in cellulose, hemicellulose, lignin and starch [4].

Lignocellulosic wastes have been used in different industrial activities including the fermentation for the biofuel production [3], the biotransformation for edible fungi [5], the development of sustainable construction materials [6] and others environmental applications such as the preparation of adsorbents for the removal of priority pollutants [7]. The last option has resulted as one of the most attractive and viable alternatives for the utilization of these wastes since the lignocellulosic biomasses offer several advantages such as wide diversity, availability in high amounts and renewability [8]. Note that the synthesis of adsorbents from this feedstock is simple due to the high reactivity of lignocellulosic materials. Besides the disposal costs and the environmental impact of waste management can be reduced with this application [9]. These biomasses have been used as adsorbents (in its raw form or chemically modified) or as precursors of carbon-based adsorbents [10,11]. However, the modification of the surface chemistry of these adsorbents can be improved significantly and, consequently, they are more stable from the chemical point of view [7,10,12].

This study focuses on the preparation of lanthanum-, cerium- and calcium-functionalized adsorbents to remove heavy metals from aqueous solutions. These adsorbents have been obtained using avocado seeds as feedstock. The fruit of the avocado is substantially consumed in the food industry (i.e., the annual production of avocado worldwide in 2017 was 6048508 tons). However, its seed that represents the 10-13 % of the fruit can be considered as an agricultural waste, which is discarded without further applications [13]. Several alternative uses have been proposed for this organic waste including its use as an adsorbent and as a precursor for the synthesis of carbon-based adsorbents for the removal of priority pollutants from water [8,13-16]. Herein, it is important to remark that the number of studies related to the heavy metal removal using this biomass is limited. Therefore, this paper aims to contribute with the valorization of such residue as a feedstock for the preparation of an adsorbent for the removal of metallic species. This type of adsorbent could be also an alternative product to be obtained in a biorefinery context.

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## EXPERIMENTAL

A carbon-based adsorbent was obtained via the pyrolysis of avocado seeds with a mean particle size of 0.50 - 0.85 mm, at 800 °C for 2 h with a heating rate of 10 °C/min and a nitrogen flow of 100 mL/min. This adsorbent was functionalized with aqueous solutions of lanthanum, cerium and calcium using a ratio of 5, 10 and 20 % w/w, respectively. These solutions were prepared using lanthanum (III) nitrate hexahydrate, ammonium cerium (IV) nitrate and calcium nitrate tetrahydrate salts plus deionized water. The mixture adsorbent – (lanthanum, cerium or calcium) solution was stirred at 25 ° C for 1 h using a rotavapor. Then, a NaOH 1.5 M solution was added dropwise until a lanthanum, cerium or calcium hydroxide precipitate was obtained. After precipitation, the temperature was increased until 70 °C and the system was stirred for 1 h. The suspension was filtered and the functionalized adsorbent was rinsed with deionized water, dried at 105 °C for 24 h and calcined during 2 h at 800 °C for the case of lanthanum- and cerium-impregnated samples, and at 900 °C for calcium-functionalized adsorbents using a nitrogen flow of 100 mL/min in all cases. Several adsorbents (M-La, M-Ce or M-Ca) were obtained varying the lanthanum, cerium and calcium concentrations and these materials were washed with deionized water.

The morphology and elemental composition of raw adsorbent was characterized by scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX) using a FEI Quanta 3D FEG equipment. The functionalized adsorbents were characterized by X-ray Diffraction (XRD) where XRD patterns were obtained at 40 kV and 45 mA with an Empyrean Diffractometer (Malvern-Panalytical) in the Bragg-Brentano geometry from 10 to 150 °2θ and using Cu-Kα radiation.

The heavy metals adsorption capacities ($q_{ads}$, mg/g) of lanthanum-, cerium- and calcium-functionalized adsorbents were determined using agitated tanks at 30 °C, pH 5, 24 h of equilibrium time and agitation speed of 150 rpm. These adsorption experiments were performed with an adsorbent-adsorbate ratio of 2 g/L and heavy metal solutions with an initial concentration of 250 mg/L. All experiments were carried out in triplicate obtaining a reproducibility of 5%.

## RESULTS AND DISCUSSION

SEM micrographs of the avocado-based adsorbent are reported in Figure 1. It showed a regular, rough and porous structure. EDX results indicated that the relevant elements of the adsorbent were carbon (92.7%) and oxygen (6.3%) and it also contained trace elements such as Mg, Al, Si and P.

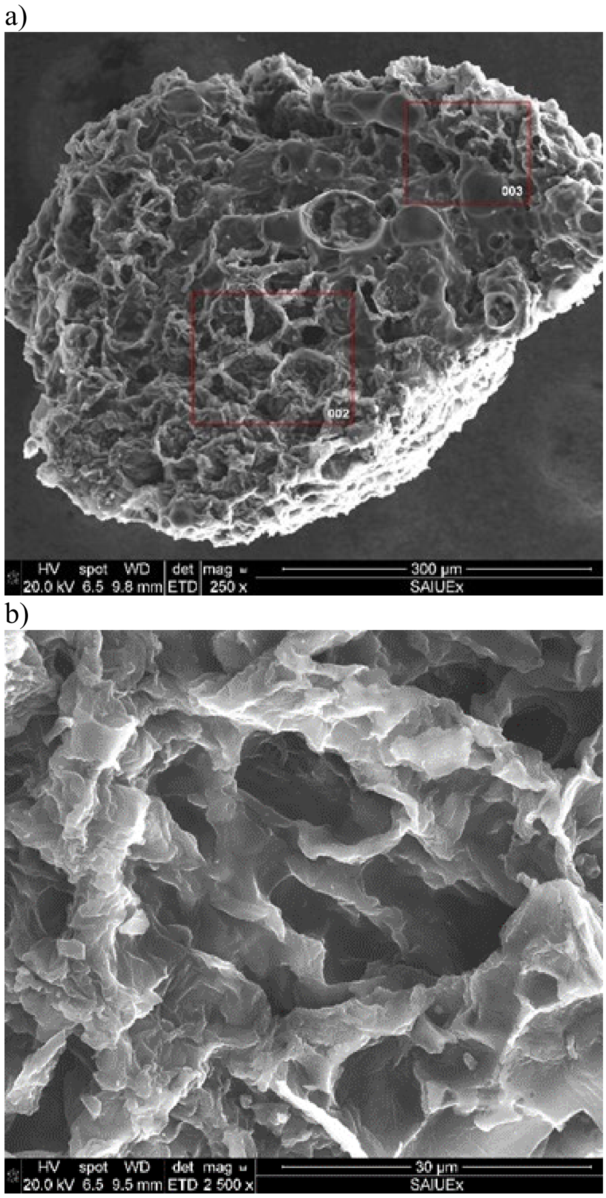

**Fig. 1.** SEM images of raw avocado seed-based adsorbent at a magnification of a) 250x and b)2500x.

Figure 2 reports the XRD patterns of all adsorbents, which showed the characteristic diffraction peaks of lanthanum hydroxide, cerium oxide and calcium oxide, respectively. This result confirmed the surface functionalization of the carbon-based adsorbents with the different elements. The crystallinity of adsorbent samples increased as the percentage of the functionalized specie increased. Note that the XRD patterns exhibited the characteristic diffraction peaks of graphitic structures at ~23 and 43 °2θ [17].

Adsorption capacities of tested adsorbents were: 2.02 - 7.05 mg/g for cadmium, 1.97 - 10.24 mg/g for zinc, 0 - 15.30 mg/g for copper and 4.14 - 18.75 mg/g for nickel, see Figure 3. In general, the adsorption capacities of nickel and zinc were higher than those obtained for the adsorbent without lanthanum, cerium and calcium, while the removal of cadmium and copper was not improved. An increment of the nickel uptake up to 70 % was obtained for the adsorbent modified with a calcium solution at 10 % (see Figure 3a). However, the adsorbents modified with the lanthanum salt showed the best performance for the nickel removal. Zinc adsorption increased up to 60 % with the surface chemistry modification using both lanthanum and cerium, see Figure 3b. Results showed that the best functionalization conditions corresponded to M-La 10% and M-Ce 20%. These findings indicated that the heavy metal properties played an important role in the adsorption process. Similar trends have been reported in previous studies for the adsorption of heavy metal ions [8,12].

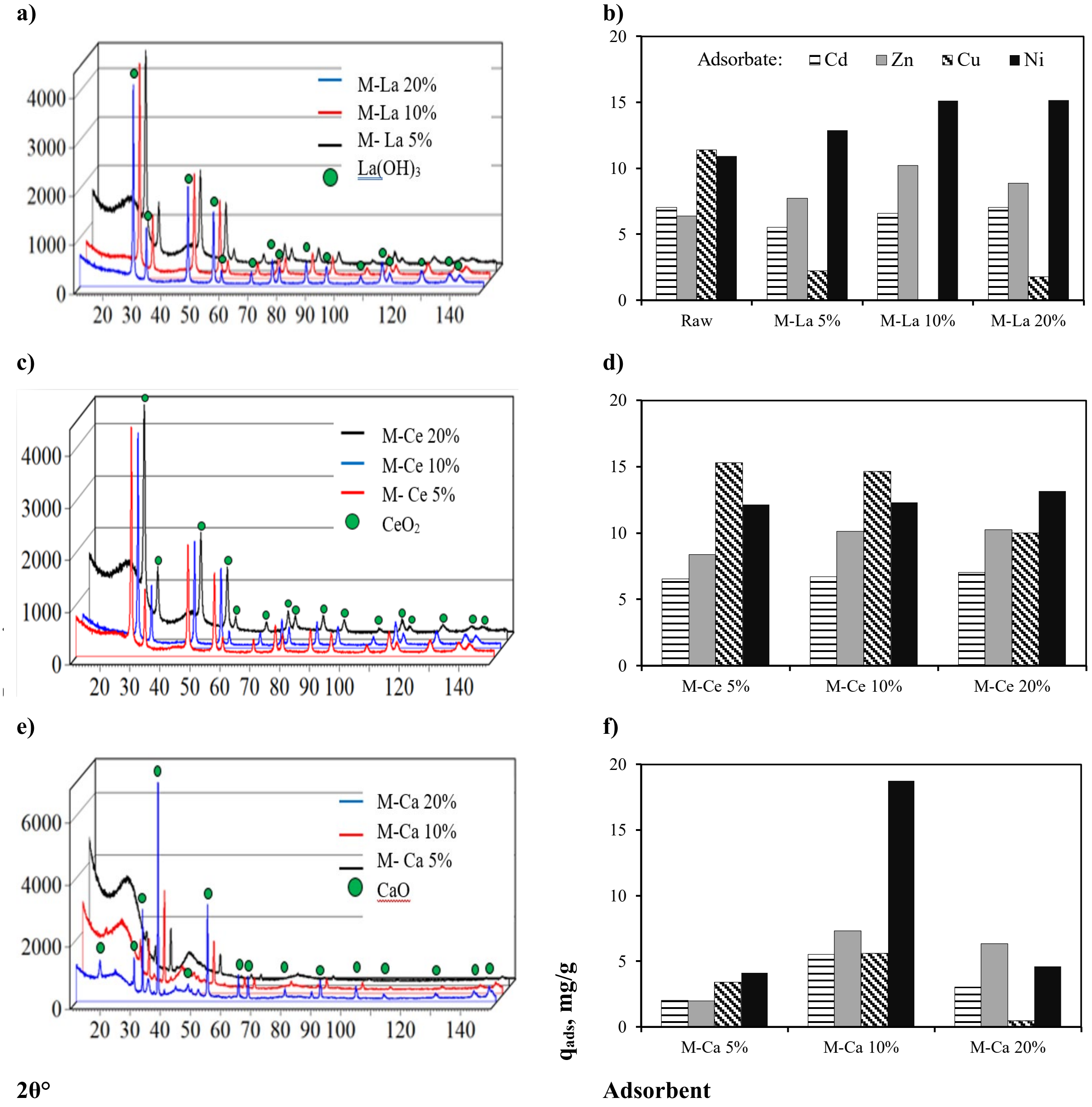

**Fig. 2.** XRD patterns of a) M-La, c) M-Ce, e) M-Ca and adsorption capacities of cadmium, zinc, copper and nickel using b) M-La, d) M-Ce and f) M-Ca adsorbents.

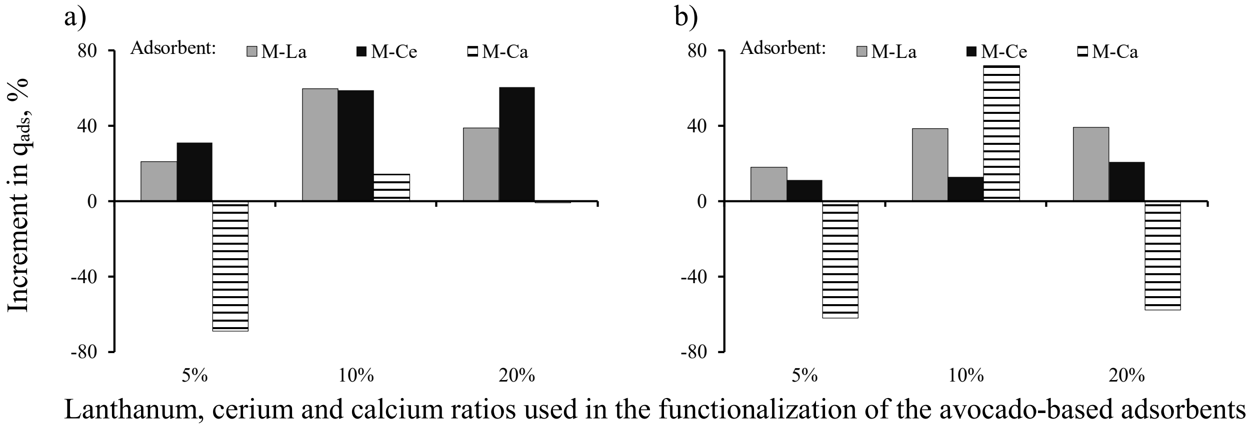

**Fig. 3.** Improvement in the adsorption of a) zinc and b) nickel using M-La, M-Ce and M-Ca modified adsorbents.

## CONCLUSION

The incorporation of lanthanum, cerium and calcium functionalities is an alternative to tailor the surface chemistry of avocado-based adsorbents to remove priority pollutants such as nickel, copper and zinc from aqueous solutions. The preparation of adsorbents or other materials useful for environmental applications contributes to the alternatives to reuse, minimize and valorize wastes generated by the food industry and, in turn, to face the water and wastewater treatment.

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# The potential use of calcium-doped flamboyant char as a heterogeneous catalyst in triglycerides transesterification

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This study reports the synthesis of heterogeneous catalysts from flamboyant biomass and their evaluation on the transesterification reaction of safflower oil with methanol to produce biodiesel. The optimization of catalyst preparation conditions was performed. Results showed that the concentration of doping metallic specie was the main variable that affected the performance of flamboyant-based catalyst in the reactive system to obtain the biofuel where the highest formation of methyl esters was 93 %. Physicochemical characterization of the carbon-based catalysts was done with X-ray diffraction and FTIR.

**Key words:** Biodiesel, heterogeneous catalysts, flamboyant biomass, transesterification

## INTRODUCTION

The increase of energy demand, fossil fuel costs and global warming have been the key factors to diversify the sources for obtaining low-cost and clean energy [1-3]. Biodiesel is an alternative to fossil fuels because of its renewability, biodegradability, ready availability, higher combustion efficiency, lower sulfur and aromatic content and higher cetane number [4]. It also has the potential capacity to satisfy the energy demand in the agriculture, commercial, industrial and transportation sectors [5]. To date, a number of studies has focused on the evaluation of methods for the biodiesel production. Transesterification of glycerides (mainly from biomass, vegetable oils or animal fats) is employed to produce biodiesel because its simplicity and cost [5-8,10,11]. Transesterification reaction is typically carried out in the presence of a catalyst [6]. The type of catalyst is one of the most important parameters in the transesterification reaction for biodiesel production. This parameter also contributes to reduce the biodiesel cost.

Heterogeneous catalysts have been widely utilized due to their advantages such as reuse and easy separation, low cost and green production [12]. Several heterogeneous catalysts have been reported for the biodiesel production. In particular, the carbon-based catalysts are attractive because of its chemical activity, thermic stability and the versatility to manipulate its physicochemical properties [13]. These catalysts can be prepared from agricultural, forest and urban wastes, which have been suggested as low cost feedstocks because of their availability [11,13,14]. To improve its catalytic activity, the support surface can be modified to tailor its acidic o basic catalytic properties using active metal species, oxidizing atmospheres, bases, acids or oxides [15-17]. It is important to remark that the selection of the precursor and the preparation route are relevant parameters to improve the catalyst performance. The preparation route involves a wide variety of possibilities to synthesize versatile, biodegradable and effective catalysts for the biodiesel production. Therefore, it has been recognized that there is still the challenge of obtaining low cost materials with outstanding catalytic properties for the biodiesel production. This article reports the preparation and evaluation of flamboyant-based catalysts and its application in biodiesel production. The impact of synthesis route on catalyst performance for the transesterification reaction of safflower oil with methanol to produce biodiesel was analyzed.

## EXPERIMENTAL

### *Preparation and evaluation of flamboyant-based catalysts*

Flamboyant pods were used as precursors to synthesize the heterogeneous catalysts. This biomass was washed, dried, ground and sieved. Heterogeneous catalyst was obtained via the pyrolysis of this biomass to obtain a porous material, which was utilized to support calcium species that acted as the active phase. An experimental design

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was utilized to synthesize 16 samples of catalysts with the aim of identifying the best preparation conditions. The variables analyzed in this experimental design were the pyrolysis temperature and dwell time, the concentration of calcium nitrate solution used to modify the surface chemistry of the flamboyant-based support and the activation temperature. All the catalyst samples were evaluated in the transesterification reaction of safflower oil with methanol. Flamboyant-based catalytic supports were synthesized at different pyrolysis temperatures (600 – 900) under $N_2$ atmosphere with different dwell times (2 – 5 h) using a tubular furnace. These supports were modified using calcium nitrate with concentrations from 0.05 to 0.40 M and different impregnation temperatures (30 – 90 °C) during 5 h at constant stirring. Finally, the calcium-doped flamboyant chars were submitted to a thermal treatment at 400 – 700 °C for 2 h under $N_2$ atmosphere. All catalysts were washed to eliminate the excess of reagents and finally dried.

The reaction conditions to evaluate the catalysts performance were: alcohol to oil molar ratio of 15:1, reaction temperature of 80 °C, 5 % wt. catalyst with respect to the oil and 5 h of reaction time using a batch reactor. After 5 h, the mixture was centrifuged in order to separate the catalyst from the liquid phase containing the biodiesel. The excess of methanol was evaporated from the solution and fatty acid methyl esters content (FAMEs, %) was analyzed by gas chromatography. A Thermo Scientific Trace 1300 GC equipped with a flame ionization detector (FID) was utilized for these quantifications. Methyl esters content was determined using the methyl heptadecanoate reagent as internal standard.

### *Physicochemical characterization of catalysts*

Selected samples of catalysts were characterized with X-ray diffraction and FTIR to analyze the surface chemistry of the flamboyant supports and their corresponding catalysts. XRD patterns were recorded with a PANalytical Empyrean X-Ray diffractometer using the configuration of Bragg-Brentano with CuKα1. FTIR spectra were obtained using a Thermo Scientific Nicolet iS10 spectrophotometer where the catalysts samples were dispersed in KBr reagent to be measured as pellets.

## RESULTS

Results of the best catalysts and their synthesis conditions are presented in Table 1. The percentages of FAMEs formation ranged from 77.3 to 90.1 %. ANOVA analysis of experimental design indicated that the calcium nitrate concentration was the main variable that impacted the catalyst performance. The best flamboyant-based catalyst was synthesized using a support obtained from the pyrolysis of flamboyant biomass at 600 °C for 4 h, which was modified with calcium nitrate at 0.4 M at 70 °C and submitted to a final thermal treatment at 700 °C. The highest FAMEs formation was 93 % of methyl esters. Kinetic studies were carried out with the best catalyst and results are reported in Figure 1.

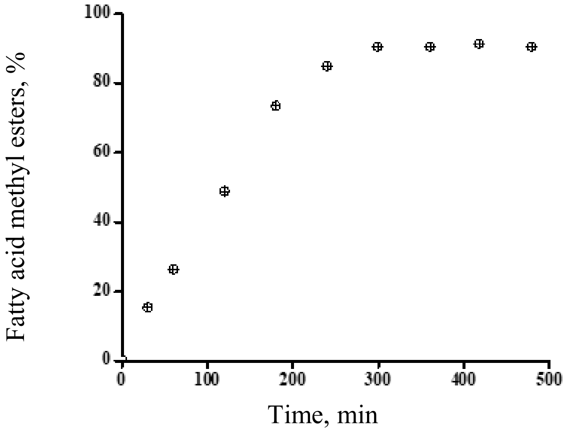

**Fig. 1.** Kinetics of transesterification reaction of safflower oil with methanol using a flamboyant-based heterogeneous catalyst.

**Table 1.** Synthesis conditions of flamboyant-based heterogeneous catalysts and their evaluation in the transesterification reaction of safflower oil with methanol to produce biodiesel.

| Pyrolysis conditions to obtain the support | | | Modification of the support using calcium nitrate | | | |
|---|---|---|---|---|---|---|
| Catalyst sample | Temperature, °C | Dwell time, h | Salt Concentration, M | Impregnation temperature, °C | Thermal treatment, °C | FAME formation , % |
| CC1 | 600 | 2 | 0.05 | 30 | 400 | 77.3±0.14 |
| CC2 | 600 | 5 | 0.40 | 90 | 700 | 90.1±0.01 |
| CC3 | 700 | 5 | 0.25 | 50 | 400 | 82.7±0.07 |
| CC4 | 800 | 5 | 0.10 | 30 | 600 | 81.3±0.28 |
| CC5 | 900 | 5 | 0.05 | 70 | 500 | 78.0±0.01 |
| CC6 | 600 | 4 | 0.40 | 70 | 700 | 93.0±0.01 |

High FAMEs formation occurred at the first 3 h of reaction where 5 h was the equilibrium time. Kinetic experimental data were fitted to the pseudo-first and pseudo-second order models according to the next equations:

$$-\ln(1 - X_{BIO}) = k_1 t \qquad (1)$$

$$\frac{X_{BIO}}{1 - X_{BIO}} = k_2 t \qquad (2)$$

where $X_{BIO}$ is the mass fraction of FAMEs, $k_1$ is the reaction rate constant in $\text{min}^{-1}$, $k_2$ is the reaction rate constant in L $\text{mol}^{-1}$ $\text{min}^{-1}$ and $t$ is the time in min, respectively. Determination coefficients were 0.97 and 0.89 for pseudo-first and -second order models, respectively. The pseudo-first order equation was the best model to describe the kinetic reaction with a rate constant of 0.0072 $\text{min}^{-1}$.

Results of XRD characterization were used for phase identification of catalytic species, see Figure 2. The diffractograms of the flamboyant-based supports showed a wide peak at 25 ° that corresponded to the graphitic structure, which is typical of lignocellulosic-based materials [18].

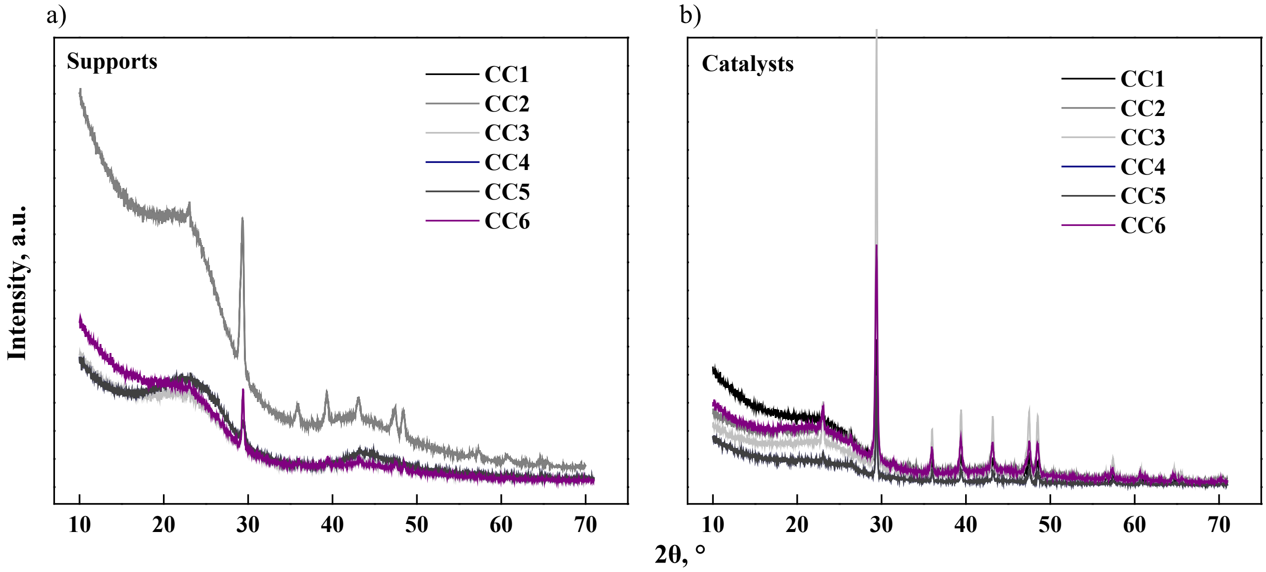

**Fig. 2.** XRD results of a) flamboyant-based supports and b) flamboyant-based heterogeneous catalysts.

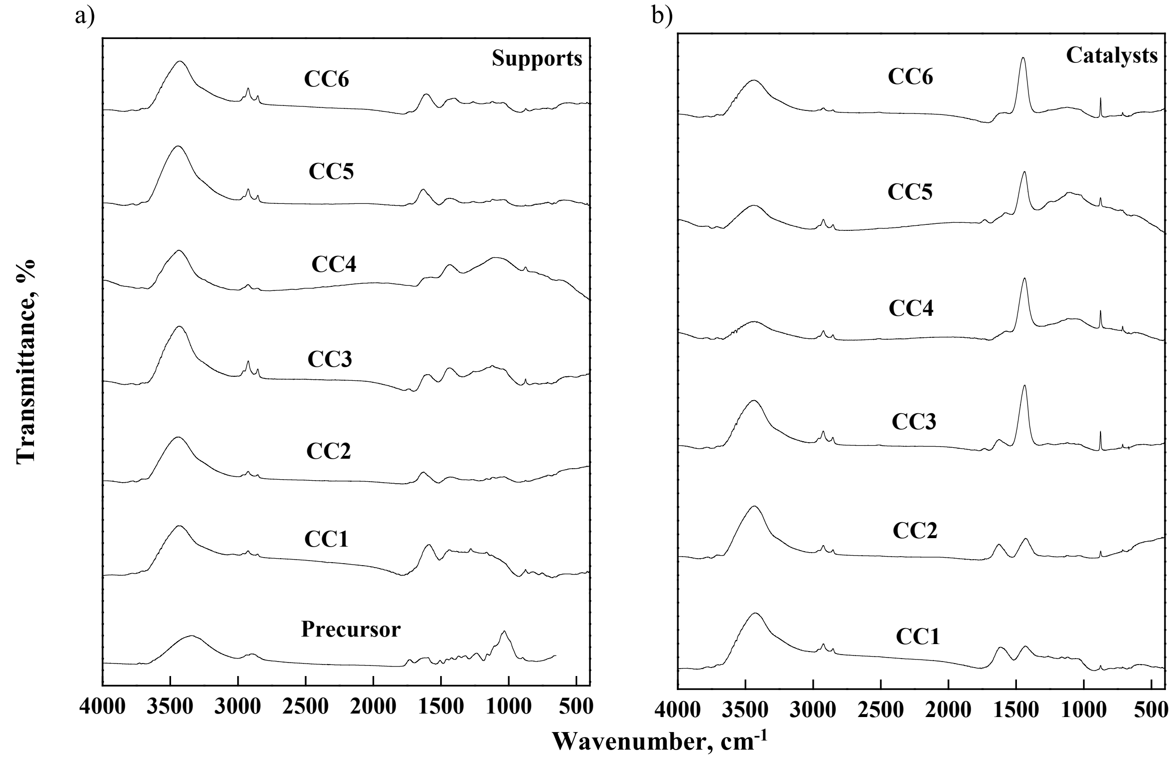

**Fig. 3.** FTIR spectra of a) flamboyant-based supports and b) flamboyant-based heterogeneous catalysts.

Catalyst diffractograms given in Figure 2b showed the characteristic peaks of $CaCO_3$ according to the PDF-2 database. FTIR results are reported in Figure 3. A band that corresponds to hydroxyl groups of phenols and alcohols [19] appeared in the region of 3500 – 3000 $cm^{-1}$ of FTIR spectra obtained for flamboyant supports. The bands corresponding to aliphatic groups were observed in the region 2916 – 2840 and 1504 – 1236 $cm^{-1}$. The bands at 1650 and 1520 $cm^{-1}$ were assigned to carboxylic groups and aromatic bonds. The bands at 1160, 1106 and 1029 $cm^{-1}$ can be attributed to the C – O vibration probably associated to acid, alcohol, phenol, ether and esters groups [20]. The band at 892 $cm^{-1}$ can be attributed to ring structures. For the catalyst samples (Figure 3b), the bands at 3500 – 3000 and 1650 $cm^{-1}$ showed a decrement in its intensity and a slight displacement, which were more remarkable as the calcium nitrate concentration and the temperature of thermal treatment increased during the catalyst preparation. The appearance of a band at 881 $cm^{-1}$ can be attributed to the presence of calcium carbonate [21]. These results confirmed the incorporation of calcium species onto the flamboyant-based support surface, which played a relevant role for FAMEs transformation.

## CONCLUSION

This report has demonstrated the importance of evaluating the route for the preparation of heterogeneous catalysts employed in transesterification reactions for biofuels production. The surface modification of a flamboyant char with calcium nitrate could be an alternative to improve the performance of the catalytic processes involved in the biodiesel production.

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# BULGARIAN CHEMICAL COMMUNICATIONS

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