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Energetic and Environmental Analysis of Different Techniques for Biomolecules Extractions

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There is an increase in the demand of natural and environmentally more sustainable products, for that the biorefinery is a very promising tool due to its capacity to develop more sustainable products. Biorefinery processes are being optimized to reduce the global energy consumption and environmental impact by increasing extraction yields. In this work, two intensification techniques, microwave-assisted extraction and ultrasound-assisted extraction, have been tested and their results have been compared with those obtained by the conventional method. The best results were obtained with microwave-assisted extraction. Another studied aspect was the selectivity of the solvent, for which three ionic liquids ([C_4C_1im][Br], [C_4C_1im][OAc] and [C_4C_1im][BF_4]) and two deep eutectic solvents (Choline Chloride:Urea (1:2) and Choline Chloride: 1,4-butanediol (1:2)) were tested. The best extraction yield was obtained with Choline Chloride:Urea (1:2). The objective of this work was the evaluation of the impact of the processes in terms of energy consumption and solvents' toxicity, in addition to the determination of the extraction process performance. For this, an analysis of energy consumption was performed, with microwave assisted extraction having the lowest consumption. For the toxicity study different parameters were taken into account, concluding that deep eutectic solvents are a good option. In conclusion, the combination of microwave assisted extraction with deep eutectic solvents can be considered the most efficient extraction method.

1. Introduction

The implementation of the biorefinery is one of the greatest challenges of this century. Due to many current environmental concerns such as Climate Change, there is an increase in the demand for natural and environmentally more sustainable products. On account of that, waste valorisation processes have become a trend in recent years, especially in chemical engineering research field. The transformation of the agro-industrial raw material, initially considered as waste or low value by-products, to manufacture added-value products is being extensively studied (Gullón et al., 2018). The concept of a biorefinery is based on technologies for converting biomass into fuels, materials and chemicals in a similar way that petrochemistry does. Lignocellulosic biomass is considered an attractive raw material for this purpose because it is available worldwide, renewable, cheap, abundant and it does not compete with food industry. However, there are still limitations, especially economic ones, to the extensive application of biorefineries. To overcome these limitations different intensification processes are being developed.
The objective of the intensification of the processes is to obtain a higher extraction yield and high quality products by reducing the amount of operating units, extraction time, raw material consumption, environmental impact, global energy consumption, cost and waste generation (Perino and Chemat, 2019), as well as improving the quality and selectivity of the solvent. Currently there are many innovative processes available to carry out extractions using green processes, such as Ultrasound, Microwave, Pulse Electrical Field (PEF), Supercritical fluids, Pressurized liquids, Supercritical water, and Thermal magnetic induction among others (Perino and Chemat, 2019). Two of the most studied non-conventional techniques are ultrasound and microwave assisted extractions. These techniques have as main objectives the increase of the extraction yield together with the reduction of extraction time, solvent consumption and energy consumption. Microwave assisted extraction

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(MAE) uses two mechanisms to heat, ionic conduction and dielectric heating (Panja, 2018), which are connected with the interaction between the electromagnetic waves with the solvent (Mena-García et al., 2019). In the case of biomass, the heating creates a rapid evaporation of the moisture, generating high pressure inside the plant cells which break these up facilitating the extraction of intracellular compounds (Yu et al., 2017). Ultrasound assisted extraction (UAE) is based on the cavitation phenomenon, which enhances cellular disruption and penetration of the solvent in the solid matrix due to compression and expansion effects on the plant cells (Yu et al., 2017). These two techniques improved the extraction of the compounds present inside the cells, some of which are considered to be high value-added compounds due to their properties; such as antioxidative, anti-inflammatory, anti-allergic, antifungal and antibacterial effects, among others (Kesarkar et al., 2009).
Another important aspect for the intensification is the choice of a more selective solvent, in order to improve the efficiency of the extraction. At this point, the principles of green chemistry must also be taken into account with the aim of carrying out more environmentally friendly processes. For these purposes, the most commonly used solvents, volatile organic solvents (VOCs), should be replaced by more environmentally friendly reagents such as water, ethanol or new modern solvents that are being studied, such as ionic liquids (IL) or deep eutectic solvents (DES). The main problem with VOCs is their toxicity not only to human health, but also to the environment, as well as the potential explosion hazard and their high impact on the greenhouse effect. (Thuy Pham et al., 2010). In order to reduce these risks more environmentally friendly solvents can be used. IL and DES, apart from being generally considered as “green solvents”, they are also known as “designer solvents”, which facilitate the selective extraction of the target compounds (Passos et al., 2014).
The main goal of this work was to evaluate the energetic and environmental impact of different extraction methods for obtaining biologically active compounds. For this, different extraction methods were studied using different solvents and the obtained extraction yield and energy consumption were compared. The environmental impact was studied analysing the toxicity of the used solvents. By comparing the obtained results, the most efficient extraction method was selected, in other words, the method with the greatest extraction yield and the lowest energy consumption.

2. Materials and methods

2.1 Chemicals

Sodium tetrafluoroborate was obtained from Fischer Scientific. VWR (BDH) chemicals supplied methanol, toluene, potassium hydroxide, sulphuric acid, acetonitrile, ethyl acetate and dichloromethane. Dimethyl carbonate, acetic acid, 1-bromobutane, silver nitrate, choline chloride, urea and 1,4-butanediol were obtained from Sigma-Aldrich. FluoroChem Ltd. supplied N-butylimidazole and methylimidazole. Ethanol absolute (synthesis grade) was obtained from Scharlau.

2.2 Raw material

Errekondo Egur-Zerra Company (Basque County, Spain) provided the Larix decidua bark. For the conditioning of the bark, it was dried until constant moisture at room temperature, cleaned by pressurised air and manually, and milled with a cutting mill (Retsch SM 2000, Germany) to obtain 0.5 x 0.5 mm particles. The conditioned raw material was stored at room temperature in a dark place until it was used.

2.3 Synthesis of Ionic Liquids (IL) and Deep Eutectic Solvents (DES)

In addition to the ethanol/water (EtOH/H_2O) (50/50 (v/v/)) mixture three different ionic liquids and two deep eutectic solvents were used for the extraction of bioactive compounds from Larix decidua bark. The selected ILs were [C_4C_1im][Br] (IL 1), [C_4C_1im][OAc] (IL 3) and [C_4C_1im][BF_4] (IL 3), and the selected DES were choline chloride:urea (1:2) (DES 1) and choline chloride:1,4-butanediol (1:2) (DES 2).These solvents were synthesized in the laboratory, using different methods for each described below. The synthesis of the ILs followed the methods described previously: [C_4C_1im][BF_4] followed the method described by (Ab Rani et al., 2011), [C_4C_1im][OAc] followed the method explained by Yoneda with slight differences (Yoneda et al., 2009), and [C_4C_1im][Br] followed the method explained by Brandt (Brandt et al., 2010) with a slight change, because the reaction was done during 24 h at 75 ºC. Regarding DES, the same method was carried out for both, mixing the reagents in constant stirring, at 80 ºC during 2 h (Wang et al., 2019).

2.4 Extraction methods

Three different extraction methods were used to compare the effects of intensification on extraction yield, conventional extraction (CE), microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE). The conditions used in each extraction are those optimised by Sillero in a previous study (Sillero et al., 2018). Briefly, all extractions were carried out with 3 g of Larix Decidua bark using a fixed solid/liquid ratio of 1/10 (w/v) and with EtOH/H_2O (50/50 (v/v)) mixture as solvent during the previously optimized conditions (see Table 1).

Before the extractions, the extracts were vacuum filtered using filter paper. The extraction yield was calculated gravimetrically and referenced to a 100 g of dried pine bark.

Table 1: Conditions and equipment used for the different extractions.

Extraction method	Time (minutes)	Temperature (ºC)	Power (W)	Equipment
CE	94	65	-	Orbital shaker (Heidolph Unimax 1010 + Heidolph Incubator 1000)
MAE	-	63	100	Open vessel microwave (CEM Discover)
UAE	95	65	-	Ultrasound bath (Elmasonic 570 H, Elma)

All the extractions with IL/H_2O and DES/H_2O mixtures were carried out following the CE with a slightly difference. Briefly, one gram of bark was used, with a fixed solid/liquid ratio of 1:10 (w/v) and a mixture of IL/H_2O or DES/H_2O (75/25 (w/w)) under the same operation conditions that has been explain for CE.

2.5 Energy consumption

For the analysis of the energy consumption of each process, an energy consumption meter (Zaeel power meter) was used. By using this equipment, the total energy consumption generated by each process is measured. The values provided by the meter are in kW/h units, and they are the sum of the energy consumption of the heating process and the reaction.

2.6 Solvent toxicity

The study of the toxicity of the solvents has been carried out with a bibliographic study. The toxicity of water and ethanol is well known for all the scientific community, but in the case of the IL and DES, it is not so clear. For the analysis of water and ethanol as solvents, already published solvent guides were consulted (GSK and Pfizer solvent guides) as well as the guide published by Prat (Prat et al., 2015). However, to understand the toxicity of the ILs and DES exhaustive search was carried out in literature to understand their properties. We have to study the toxicity of the ILs and DES used and it is important to take into account the characteristics of the synthesis of these solvents. ILs and DES are often considered as “green-solvents”, but there is a need to analyse the risk associated to their productions.

3. Results and discussion

3.1 Extraction yield of the different extraction methods

As the solid/liquid ratio used in all extractions is the same, a real comparison can be made of other effects such as intensification or efficiency and/or toxicity of the solvents used.
In this study, different extraction methods and solvents were used to obtain extracts from Larix decidua bark. This was done in order to analyse the extraction capacity not only of the different methods, but also of the selected solvent. For this purpose, three methods (CE, MAE and UAE) and six different solvents were selected. Figure 1 summarizes visually the extraction yield measured for each extraction, which are very different.
The extractions carried out with molecular solvents (EtOH/H_2O) have lower extraction yield regardless of the extraction method used. MAE gave the best extraction yield, closely followed by CE. UAE, on this occasion, proved to be less effective in obtaining extracts compared to the other methods. This may be because the parameters studied for the UAE are not adjusted correctly due to the limitations of the equipment used to carry out these reactions. To improve this performance, the ultrasonic bath could be replaced by a direct application of ultrasound using a sonotrode.
Regarding the use of more selective solvents, IL and DES, can be observed to give an improvement of the extraction yield with all of their measured values at least double those obtained with the molecular solvents. This appears to be due, in the absence of verification through more thorough characterization, to a greater selectivity of the solvents. In this case, the results obtained by the DES are superior to those measured for ILs, which allows us to conclude that the selected DES are more efficient under the selected conditions. IL 1 is the one with the lowest measured extraction yield, closely followed by IL 3, while DES 1 is the one with the highest value, with a difference between them of more than 6%. However, the optimum conditions for each solvent may be different than those used here, which may influence the results.

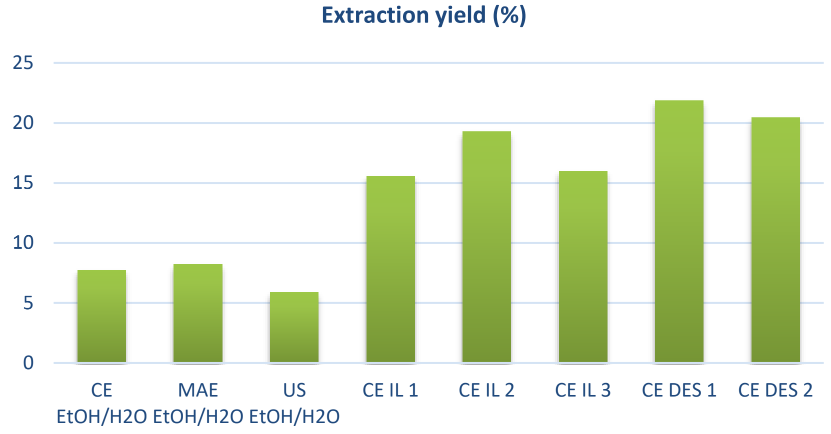

Figure 1: Extraction yield obtained for all the different extractions from Larix decidua bark carried out

Taking into account all the information mentioned above, it can be concluded that the modern solvents, ILs and DES, are more efficient in obtaining extracts from the pine bark. Joining this observation with the best yield obtained by using the MAE as the extraction intensification process, it is concluded that it would be convenient to carry out extractions with the microwave assisted extraction and using DES 1 as solvent, due to its greater extraction efficiency.

3.2 Energy consumption

One of the most important factors to consider a "green" process is the impact generated by it due to energy consumption. For this reason, the study of the energy consumption of each treatment has been carried out. In Table 2 the energy consumption for each extraction treatment versus the obtained extraction yield can be seen. After performing an analysis of the data, it has been concluded that the solvent used does not affect the energy consumption in this case. This is because not all the processes have been optimized, since the extractions with ILs and DES have been carried out by replicating the optimal conditions used for CE. This has been decided to do in order to be able to carry out a real study of the effect of these solvents on the extraction yield without having any type of interference, such as microwaves or ultrasounds.

Table 2: Extraction yield and energy consumption for each of the extractions made to the Larix decidua bark

Extraction method	Solvent	Extraction yield (%)	Energy consumption (Kw/h)
CE	EtOH/H_2O	7.7*	0.65
MAE	EtOH/H_2O	8.2*	0.33
UAE	EtOH/H_2O	5.9*	0.54
CE	IL 1	15.2	0.65
CE	IL 2	19.3	0.65
CE	IL 3	16.0	0.65
CE	DES 1	21.8	0.65
CE	DES 2	20.0	0.65

*Extractions done following the method described by Sillero et al. (Sillero et al., 2018).

It is already known that CE is a high energy consuming technique and the obtained results confirmed this fact. Below this technique is the UAE, with a lower consumption of 0.11 kW/h. MAE is the extraction technique with the lowest energy consumption, apart from having the highest extraction yield comparing between the extraction carried out using EtOH/H_2O. In the case of the UAE, an also for CE, the used system was not the most energy efficient. The problem in both cases was the necessity of heating more space/water than was really needed for the reaction. In the case of the UAE there is an added difficulty to maintain constant temperature, since it is an open bath. However, MAE is a green alternative due to its energy efficiency and higher extraction yield. In addition, the industrial scale-up approach is already taking place due to its low cost and operational simplicity

(Mena-García et al., 2019). For this last reason, the MAE is one of the best options to replace the current extraction methods.

3.3 Toxicity of the solvents

To analyze the toxicity of the different substances, the EtOH/H_2O mixture is the first. For analysis, each solvent will be studied separately. Starting with water, it is considered the most recommended and green solvent in all the consulted solvents guidelines. Its use is highly recommended. In the case of the EtOH, the classification is not so clear and it depends on the guide that is used. In some cases, it is considered totally "green" and in other, somewhat less “green”. This is mainly due to its risk of flammability that affects the safety of the users. In conclusion, it could be said that EtOH/H_2O mixture is an environmentally friendly solvent.

On the other hand, for the study of the rest of the mixtures used, it is generalized that the use of water is adequate, not only because it is a green solvent, but also because it improves the viscosity of both IL and DES, facilitating the extraction. Regarding the ILs, these substances are usually considered as “green solvents”, mainly due to their low volatility, which is summarized in almost no risk of flammability and atmospheric contamination (Hospido and Rodríguez, 2019). However, it is a mistake to consider only with this characteristic that ILs are environmentally friendly. To be able to affirm this, it is necessary to take into account other factors such as their toxicity in water, humans or soil, as well as their biodegradability and their full life cycle analysis (LCA).

IL´s biodegradability is poor; they can be accumulated in water or soil. The toxicity of imidazolium based ILs in aquatic environments is confirmed (Płotka-Wasylka et al., 2017), with a higher toxicity for IL 3 than for IL1 (Frade and Afonso, 2010). In the case of terrestrial toxicity, it is also verified that not all the ILs are toxic, but according to Frade, IL 3 has an effect on the growth of some bacteria presented in the soil (Frade and Afonso, 2010). For the other two ILs no results were found.

Focusing on the LCA, first it must be said that it is difficult to carry out the LCA for ILs or DES, since there is a lack of data. At this point, the synthesis, use and degradation of ILs in ecosystems are taken into account to understand their real impact. Making a theoretical analysis of the synthesis process, it can be said that this may be a very limiting factor to consider these as a “green solvent”, since it must comply with the principles of green chemistry. It can be said that IL 2 is a high energy consuming product, so its impact is considered to be high, the same for IL 3. The synthesis can also be a high solvent consumption step, more if high purity ILs are needed. The reactions done at this work are not very high time and energy consuming, so there will not be a high impact at this step. However, it must be taken into account that in case of wanting to purify the extracted compounds, the use of VOCs will be required, so this impact must also be taken into account, which is not the case. Finally, the effect of the ILs in the ecosystems is needed. It is closely related with the toxicity explained above, and with the biodegradability. The two best properties for the use of the ILs in the industry, thermal stability and non-volatility, are potential problems with degradation or persistence in the environment as has been reported by Thuy Pham (2010). In the same work, the effect of both the anion and the cation on biodegradability is discussed. The conclusion is that the use of oxygen-containing functional groups, such as acetate, makes it easier to degrade, and the case of the halides, they are more stable, so les biodegradable, with $[BF_4]^-$ being worse than $[Br]^-$. It also concludes that increasing the alkyl chain leads to an increase of the biodegradability. Taking into account all the above, it can be said that processes using ILs could have a higher life-cycle environmental impact than other conventional methods (Zhang et al., 2008). It is always advisable to analyze each case separately.

In the case of DES, there are limited studies, so their classification becomes more difficult. In our case of study, all the reactants used for the generation of the DES are natural, so they can be from renewable resources, having a less environmental impact (Singh and Savoy, 2020). They are also considered non-toxic, biodegradable and with good bio-compatibility (Płotka-Wasylka, 2017).

4. Conclusions

In this work it has been demonstrated that both the extraction technique and the solvent used have a considerable influence on the extraction yield from Larix decidua bark. Regarding the extraction process, the best results were obtained with MAE, in addition to being the technique that generated the least energy consumption. For the greenness of the process, it should be taken into account not only the intensification process, but also the used solvent, which should be non-toxic, biodegradable and with a high recyclability.

For this reason, the best solvent to use would be EtOH/H_2O or DES. To choose between these, the analysis of the extraction yield has carried out. In this case, it is clear that the use of DES for extractions is better since its yield is significantly higher. With all this, it is concluded that the most efficient and environmentally friendly process would be the combination of MAE with DES 1. With this combination, an extraction method which fulfills two of the principles listed for green chemistry (energy saving and the use of “green” solvents) is designed. The optimization of this extraction is pending as future work.

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