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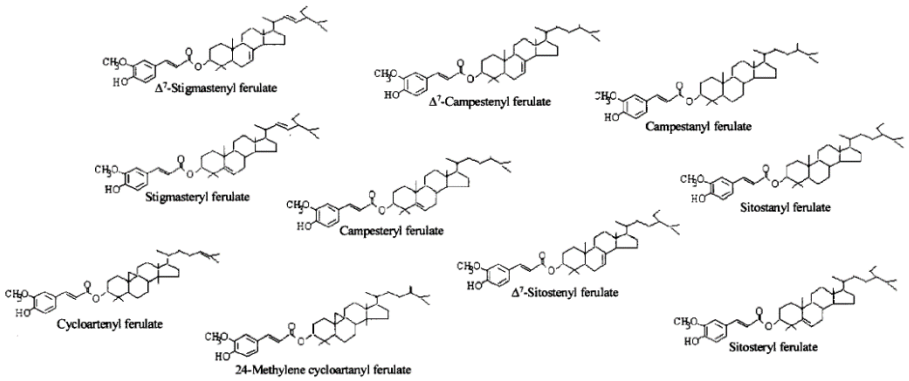

Sustainable Valorization of Rice Straw Using Deep Eutectic Solvents

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I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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Abstract

This thesis project consisted in the development of a sustainable valorization process for rice straw as it is an important biomass residue in Portugal, due to its high production. Rice straw not only is one of the agricultural residues with the biggest biofuel producing potential that can help face the global energetic crisis, but also a material rich in added value compounds, namely gamma-oryzanol (OZ) that is known for its anti-oxidative and anti-cancer properties. The process consisted in two stages wherein the first one, a liquid-liquid OZ extraction was carried out using two hydrophobic deep eutectic solvents (DES) based on octanoic acid (C8) and a common volatile organic solvent, hexane, was used as well for comparison purposes. High performance liquid chromatography analyses of the extracted phases allow to conclude that the extraction of this compound (OZ) was not successful in both cases, probably due to the very small concentration of this compound in the chosen biomass. As for the process's second stage, it involved a treatment of biomass with hydrophilic DESs based on choline chloride (CC) with the aim of solubilizing the rice straw and subsequently depolymerize it to obtain products rich in saccharides and polysaccharides, that can be further fermented to produce bioethanol. The results showed that the DES composed of CC and urea in a molar ratio of 1:2 managed the best solubilizing performance with an average recovery of 48% of the added biomass, conducing to a solubility of 25.9 mg of rice straw/g DES. In respect to the selectivity towards glucose and xylose, the DES comprised of CC and acetic acid in a 1:2 molar ratio achieved the best results. The water's effect in the biomass treatment was also studied and an optimization of this stage warranted the use of the CC and acetic acid DES in a 4 hr biomass incubation for achieving the most promising results.

Keywords: Deep eutectic solvent (DES), rice straw, gamma-oryzanol, valorization, green chemistry

Resumo

Neste projecto desenvolveu-se um processo de valorização sustentável para a palha de arroz, uma vez que Portugal se encontra no topo da lista dos maiores produtores deste cereal da Europa. Assim, a palha de arroz é não só um dos resíduos agrícolas com mais potencial para produzir biocombustíveis e ajudar a fazer face à crise energética actual mas é também um composto rico em diversos químicos de valor acrescentado, nomeadamente em gamma-orizanol (OZ) que é conhecido pelas suas propriedades antioxidativas e anticarcinogénicas. O processo consistiu em duas etapas, na primeira das quais se realizou uma extracção líquido-líquido de OZ utilizando dois solventes eutécticos profundos (DES) hidrofóbicos à base de ácido octanóico (C8) e um solvente orgânico volátil comum, hexano, para fins comparativos. As análises de cromatografia líquida de alta eficiência efectuadas às fases extraídas permitiram concluir que a extracção deste composto (OZ) não foi verificada em ambos os casos, provavelmente devido à baixa concentração deste composto na biomassa escolhida. Quanto à segunda etapa do processo, consistiu num tratamento da biomassa com DESs hidrofílicos à base de cloreto de colina (CC) com o âmbito de solubilizar a palha de arroz e consequentemente despolimerizá-la para obter produtos ricos em sacarídeos e polissacarídeos, de modo a que possam ser posteriormente fermentados para se produzir bioetanol. Os resultados mostraram que o DES composto por CC e ureia num rácio molar de 1:2 demonstrou um melhor desempenho na solubilização da biomassa com uma recuperação média de 48% da biomassa adicionada, levando à solubilidade de 25,9 mg de palha de arroz/g DES. No que toca à selectividade em glucose e xilose, o DES composto por CC e ácido acético num rácio molar de 1:2 obteve os melhores resultados. O efeito da água no tratamento da biomassa também foi estudado e uma optimização deste passo justifica o uso do DES composto por CC e ácido acético numa incubação da biomassa de 4 hrs para garantir os resultados mais promissores.

Palavras-chave: Solvente eutéctico profundo (DES), palha de arroz, gamma-orizanol, valorização, química verde

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List of Symbols and Abbreviations

DES	Deep eutectic solvent
HBD	Hydrogen bond donor
HBA	Hydrogen bond acceptor
IL	Ionic liquid
hr	Hour
min	Minute
s	Second
Å	Angstrom
µm	micrometer
mm	millimeter
cm	centimeter
ha	hectare
km^2	square kilometer
K	Kelvin
^oC	Degree Celsius
mol	mole
kton	kiloton
kg	kilogram
g	gram
mg	milligram
L	Liter
mL	milliliter
µL	microliter
rpm	Revolutions per minute
Ppm	Parts per million
Ppmv	Parts per million in volume
wt%	Weight percentage
v%	Volume percentage
CC	Choline chloride
FRU	Fructose
AA	Acetic acid
U	Urea

PEG 200	Polyethylene glycol 200
LA	Lactic acid
MA	Malic acid
OA	Oxalic acid
TA	Tartaric acid
CA	Citric acid
C8	Octanoic/caprylic acid
M	Menthol
C12	Dodecanoic/lauric acid
OZ	Gamma-oryzanol
OZ A	Cycloartenyl ferulate
P	Proline
FTIR	Fourier transform infrared spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
HPLC	High performance liquid chromatography
UV	Ultraviolet
vis	Visible
HFCs	Hydrofluorocarbons
CO_2 (eq)	Carbon dioxide equivalent
GWP100	100-year time horizon global warming potential
IPCC	Intergovernmental Panel on Climate Change
RBO	Rice bran oil
NREL	National Renewable Energy Laboratory
SFE	Supercritical fluid extraction
ASL	Acid-soluble lignin
AIL	Acid-insoluble lignin
MCC	Micro-crystalline cellulose
HMF	5-hydroxymethyl furfural
CrI	Cellulose Crystallinity Index

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1 Introduction

1.1 Sustainability

Since the start of the industrial revolution in the late 18th century with the textile industry[1], the rate of the world's population growth has increased tremendously, leading to a depletion of natural resources to satisfy its needs, accompanied by the degradation and pollution of several ecosystems. These matters were mostly ignored throughout the 18th and 19th century and only in the 20th century did they become concerns, just after major pollution incidents took the world by surprise.

Nowadays, it is important for mankind to turn the tide on ecological degradation and manage to get the most out of nature with minimal impact. The ecological footprint is a concept that arises from this philosophy as it is a measure of human demand on nature and can be estimated by taking an ecological accounting system to determine the quantity of nature necessary to support people or an economy[2]. As of today, the human population on earth consumes approximately 1.7 earths of resource each year meaning we are consuming more resources than planet earth can produce, inevitably depleting our energetic stocks[2] and reminding us that the concern for life's ecospheres has yet to grow and become a major political topic.

Another series of concepts and notions were set up such as, for example the «carbon footprint», which is the amount of carbon dioxide and methane emitted by a defined population, system or activity framed in their spatial and temporal boundary[3] , or «sustainability», which is the process of maintaining change in harmony with a balanced environment such as to enhance both current and future potential human needs and aspirations[4]. This latter concept involves several interconnected domains of the economic and social spheres as well as the environmental sphere[5] and gives birth to the idea of «sustainable development», which is the organizing principle for meeting the demands of both human development and the ability of natural systems to sustain themselves, in order to provide the resources upon which the economy and society depends, in other words it is the «holistic approach that leads us to the end point of sustainability»[6].

Along with the sustainability definition, other concepts were put forward such as, for example, biodegradability which has been sought to guarantee that the amount of waste is minimized by using organic matter that can be decomposed by microorganisms. This process takes place in three steps, the first being biodeterioration, where a more superficial decomposition occurs and a change in mechanical and chemical properties of the material is caused by environmental factors, namely weather, external mechanical deformations and the exposure to chemical elements. the next step is biofragmentation, where the cleavage of polymeric bonds happens at the hands of microorganisms, creating oligomers and monomers to be integrated in the last biodegradation's step, called assimilation[7]. This mechanism allows for a clean and efficient way to dispose of organic materials and composting is one such example of these advantages; it is a biodegradation process that occurs in a controlled environment to assure a specific set of conditions, often used in wastewater's sludge treatment[8] and as a solid waste disposal technique in general, to transform waste products into an organic soil amendment[9].

Nonetheless as for some materials the notion of biodegradability is not applicable, there is the need to implement the concept of recyclability that has also been an important answer to ensure a brighter future for it not only reduces the consumption of natural resources, since we can now use the recycled material in the creation of newer objects, but also takes care of an ever increasing waste production. In fact recyclability and biodegradability go hand in hand as the aforementioned example of composting shows, considering it is fundamentally the recycling of organic solid waste for producing fertilizers.

Yet, it must be remarked that despite the low ecological footprint, these processes still have an associated carbon footprint as it is practically indissociable from any activity and processes as trivial as aerobic or anaerobic digestion, that take part in the biofragmentation step of biodegradation[7], emit greenhouse gases, carbon dioxide and methane respectively, that in excess can contribute to global warming so it is interesting to note that just because a method has a lower ecological footprint does not necessarily mean that it will have a proportionally lower carbon footprint as less of the earth's resources may be used in the product's manufacturing but now that a new, more complete, process is in place, there will be the need to supply an additional amount of energy to this new optimization which in turn causes an emission of greenhouse gases; to achieve sustainable development is a difficult matter and it is necessary to tackle all these indicators at once to be successful, hence the efforts in establishing a relationship between the many variables and correlating them with one another.

In a similar attempt, it has been shown that by studying as much as 30 multi-metric indices for evaluating sustainable development, it is possible to reduce them into 5 canonical factors that explain a significant amount of variation; the two first factors, being «prosperity, equality & governance» and «quality of life», pertain to a set of anthropocentric characteristics that comprise affluence, social capital, optimism in governance while being associated with a nation's peacefulness and negatively affected by government corruption and income inequality while the other three factors, «ecosystem integrity», «environmentally efficient happiness» and «environmental management» are directly linked with the environment well-being and strongly connected to human made policies concerning the biosphere[6].

Regarding these policies and maintaining a more pragmatical approach, efforts to regulate the carbon footprint in a worldwide scale have culminated in the Kyoto Protocol; it is an international treaty that follows the United Nations Framework Convention on Climate Change and was adopted five years later, in 1997, to carry out its initial plan of stabilizing atmospheric greenhouse gas emissions to avoid manmade interference to climate systems and its consequences[10] and while the original treaty did not have any mechanisms nor stipulated limits to enforce the commitment[11], the Kyoto Protocol on the other hand, specifies the emission's reduction amount and establishes a compliance committee with delegated instructions for the non-compliant ratifying parties to meet their goals, as well[12]. It must be defined that a greenhouse gas is a gaseous compound at atmospheric pressure that absorbs the infrared wavelength, from 4 to 100 μm, emitted by the earth's surface and is in turn excited as this long-wave energy corresponds to their vibrational-rotational spectrum. After this first absorption step, thermal long-wave photons' emission by these compounds in rotational or vibrational

excited states follows, warming up the atmosphere as half of the thermally emitted photons go back to the earth's surface[13] instead of being allowed to freely exit the earth's atmosphere into space; it is essentially a «gas that absorbs and emits infrared radiation» and thanks to their presence, the earth's surface is 15^{o}C hotter than it would be without them[14].

The greenhouse gas emissions' reduction covered by this agreement are those of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons and sulphur hexafluoride[10], measured collectively in CO_2 (eq), with the first three substances being the most important in the modern atmosphere due to the global industrialization of the past century accelerating their emission rate by reason of being especially linked to the combustion of fossil fuels and biomass; from these three, carbon dioxide is the most prominent with annual emissions of over 40 billion tons and although atmospheric CO_2 can be diluted via several mechanisms as being fixed by plants, both on land and in water, partly dissolved into bodies of water and integrated into some rocks in geochemical time spans[15], they have not sufficed to contain the additional emitted amounts, as CO_2 records in the oceans from 1996 to 2007 have shown, along with ice-core analysis that quantify its concentration, to have been around 280 ppmv in 1750 and 310 ppmv in 1950 leading to 380 ppmv at the start of this decade as observed by a ground station of the Intergovernmental Panel on Climate Change (IPCC) at Hawaii[15]. As for the CH_4 atmospheric concentration, it is nowadays around 1,8 ppmv with a slight increase compared to the eighteenth century, where estimations of 0,8 ppmv were made, mostly due to leakages from the processing of fossil fuels as it is a major component of natural gas and widely used as a clean fuel and as for the N_2O atmospheric concentration, it is around 0,32 ppmv, also estimated to have increased by at least 10% from the preindustrial era because of its augmenting emissions from a growing agricultural sector whence it is closely connected to synthetic fertilizers[15] and created as a result of the interactions between organic material, nitrogen and moisture[16]. It must be pointed out that even though these two last compounds are present in diminute amounts, they have a superior 100-year time horizon global warming potential (GWP100), as established by the IPCC, meaning that 1 mol of CH_4 or N_2O has a bigger impact on the atmospheric temperature rise than 1 mol of CO_2 as it is a measure of the cumulative radiative forcing over the chosen time horizon caused by the compound, relative to the reference substance, CO_2, in other words, it is how much heat a greenhouse gas is capable of trapping in the atmosphere for a 100-year time horizon, compared to CO_2 that has a GWP100 of 1[17]; in fact CH_4 has a GWP100 of 28, N_2O has one of 265 and chemicals like HFCs, that were created to substitute the ozone-depleting chlorofluorocarbons, have GWP100s that range from 4 to 12400 whilst sulphur hexafluoride has the highest value of 23500[18]. Although there are other metrics, with each one serving a precise purpose or a certain application, the Kyoto protocol adopted this one alongside the measure of CO_2 (eq) allowing these anthropogenic climate agents to be discussed and expressed in a common unit in order to facilitate the handling of these multi-component climate policies[17].

In respect to the Kyoto protocol's objectives fulfillment, much could be said as 3 years after its first commitment period started, more than a decade after the original treaty was adopted to give enough time for the ratifying parties to prepare[10], the vast majority of European countries were on track towards their

respective Kyoto targets and achieving their goals[19]; by the end of the 4 year commitment, in 2012, 36 out of the 37 parties managed to comply with their objectives[20] with the parties to the protocol surpassing their agreement amounts by an average of 2,4 Gton CO_2 (eq) per year[21], thus showing at first sight that climate policies, although hard to implement, can succeed after all. On a closer look though, the situation takes a more discouraging turn because as many major emitters did not ratify the protocol, the total emissions covered by the treaty only amount to 18% of global emissions[22] and the fact that superpowers like the US and China, responsible for emitting over 18 Gton CO_2 (eq) in 2012[23], did not ratify the agreement or were exempted from it[24], overshadows the accomplished parties' feats, specially with statements mentioning how putting the treaty in place would harm developed countries' national economies and how it was a «bad deal»[25].

In December 2012 a second commitment phase was agreed upon, right after the end of the first commitment period, and discussions led to a deal being struck by nearly 200 nations to extend the Kyoto Protocol until 2020 but in which only Europe and Australia with a global greenhouse gas emission share of less than 15% have legally-binding objectives[26], more so, the Paris agreement that ensued 3 years later from this deal and that came in as a replacement for the aforementioned protocol[24] leaves each country to determine and plan their own actions to mitigate global warming for limiting global temperature rise to «well below» 2^{o}C above pre-industrial levels[27] but with no specific targets and no specific date[28]. Although it encourages parties to be ambitious and «pursue their best efforts through nationally determined contributions»[29] by giving room and flexibility for each country to decide how they should tackle the adversity, as of July 2017, none of the major industrialized countries are meeting their pledge to cut on greenhouse gas emissions and only when the formal reviewing process will begin, around 2020, will we have concrete evaluations[30].

As such, one of the challenges of this century has been to find ways to respect these goals and notions which involves using sources of energy and materials that help us achieve them; renewable and clean energy sources are therefore the key to a better future as societies' dependence on fossil fuels, that come from non-renewable sources[31], is one of the factors that accompanied the industrial revolution and not only helped to increase the rate of global warming due to the associated anthropogenic climate agents' emissions but also led indirectly to the major pollution incidents that shook the world. Despite this, if we take a look at the worldwide energy supply in 2010, non-renewable energy sources account for 87% with fossil fuels contributing 81%[32] and in the case of one of the biggest superpowers in the world, the United States (US), we can see how much these fuels are still part of modern societies' habits since in 2016, 76% of all emissions with global warming potential in the US arose from fossil fuel combustion[33], showing us that the trend continues. Nevertheless it must be pointed out that this problematic matter goes deeper than the usage of fossil fuels. In 2012 this same superpower had a CO_2 (eq) emission of 6,3 Gton CO_2 (eq)[23] from which 5,1 Gton were directly from CO_2 emissions[34] and where gaseous, liquid and solid fuel consumption contributed with 1,3 Gton[35] , 2 Gton[36] and 1,6 Gton[37], respectively, making up over 96% of the total CO_2 emissions and over three quarters of all US greenhouse gas emissions in this same year and yet in every single fuel consumption mentioned above, a renewable, cleaner energy source is also associated. For the solid fuel fraction, for instance, we can account for the combustion of biomass and for the liquid fuel fraction we also have the consumption of biodiesel, that is a

vegetable oil-based fuel[38] produced by their transesterification and esterification with an alcohol[32], usually methanol[31], making biodiesel being defined as «a fuel comprised of mono-alkyl esters» by the National Biodiesel Board[39], meanwhile concerning the gaseous fraction we have biomethane that constitutes natural gas and comes from the anaerobic fermentation of organic matter[40]. This shows, paradoxically, that while renewable energy sources are promising and cleaner[40] and should recruit more investment, their application might not be dissociated from harmful effects to the earth's ecospheres as it might impose changes in an unharmonious way, remembering us that although these new technologies are helping us achieve sustainable development, we should not limit our research and development while always striving to resolve the ever growing energy needs of an ever growing society.

These alternative fuels obtained from renewable sources, commonly named biofuels[41], are obtained from biorefineries that, in an analogous way to a standard petroleum refinery, produce fuels, power and chemicals by integrating biomass conversion processes and technologies[42] by using the principles of green chemistry to fulfill sustainability and in turn ensure a longer lifetime to the process as its resources and their cycles are preserved[43].

1.2 Green Chemistry and Biorefineries

Green chemistry is a field of chemistry and chemical engineering that focuses on designing products and processes that are environmentally conscious and minimize the use and generation of hazardous substances[44]. It is an ever expanding discipline that is governed by 12 distinct principles: prevention, atom economy, less hazardous chemical syntheses, design for safer chemicals, safer solvents and auxiliaries, design for energy efficiency, use of renewable feedstocks, reduction of derivatives, catalysis, design for degradation, real-time analysis for pollution prevention and inherently safer chemistry for accident prevention[45]. As chemical engineering is under tremendous pressure from regulation protocols like REACH (Registration, Evaluation, Authorization and Restriction of Chemicals[46] and RoHs (Restriction of Hazardous Substances[47] to change not only many of the harmful chemical substances used but also the less efficient and waste generation processes, leading to increased efforts in finding environmentally friendly substitutes to the compounds we use nowadays, such as HFCs, volatile chlorinated solvents , aluminum chloride in organic processes, among many others[41].

In the 21st century, chemical engineering is faced with many major challenges being some of the most important the depletion of the fossil fuels, the continuous increase of CO_2 production with its release to the atmosphere and the non-sustainable energy demand of the modern life. The birth of biorefineries has been prompted to answer these problems mainly due to their renewable nature. The dilemma of food versus energy introduced by the biorefinery concept was soon overcome and nowadays the use of waste produced from other industries, namely the food industry, where approximately one-third of all produced food is wasted, and the agriculture and forestry industry, has allowed a new breath of biorefinery[41]. The following figure sums this novel concept of biorefineries as their main resources can be waste from other industrial fields.

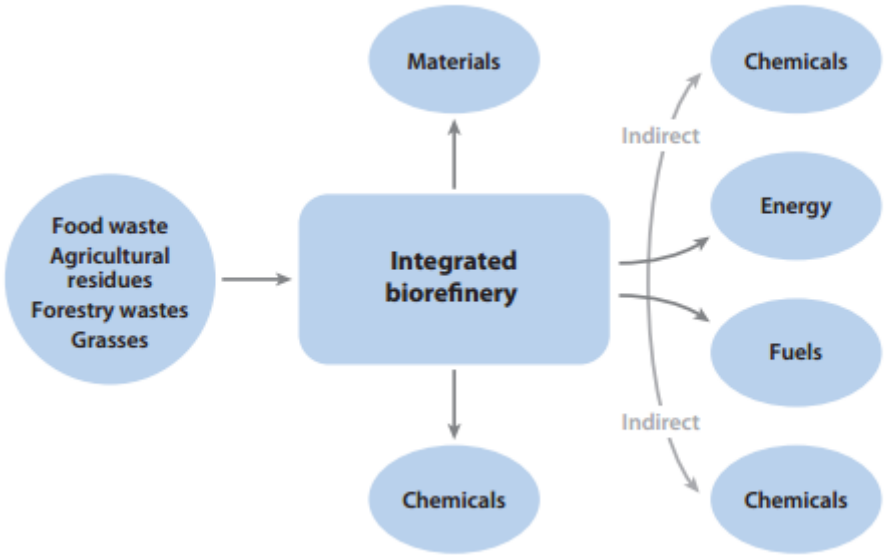


Figure 1 - The integrated biorefinery as a mixed feedstock source for chemicals, energy, fuels and materials[41].

The objective is to manufacture high-value low-volume products (HVLV), that are largely profitable, and simultaneously to add value to the industrial process with low-value high-volume (LVHV) products that can be converted into energy to meet the global energy demand through a network of processes[48]. These technologies that can be classified as extraction, biochemical and thermochemical processes where in these last two, the extraction of valuable chemicals is sought for to increase the overall financial return[41]. While the former processes operate at lower temperature and higher selectivity, normally requiring preprocessing steps, to prepare the biomass for the extraction of HVLVs, that usually are time consuming and lead to difficult downstream processing units like distillation that lowers the process's energy efficiency[41], thermochemical processes on the other hand involve higher temperatures (>500^{o}C) in procedures that include pyrolysis, which is the decomposition of organic matter under an oxygen reduced atmosphere into biochar and bio-oil[49], gasification to produce syngas[48], and direct combustion for the production of oil, gas, char or ash and that also reduce the energy efficiency of the facility while being nonspecific. Consequently, the combination of both types of processes is paramount to assure significant advantages[41].

Biorefineries can be divided into 3 different types/phases as they progress and develop. Facilities referred as phase I have limited processing capabilities and only manage to convert a single feedstock into a single major end product. Examples of Type I biorefineries include biodiesel plants, where rapeseed or sunflower oil, obtained by crushing the seeds, is transformed into biofuel[41] via the aforementioned process, and dry mill ethanol plants for instance.

As for Type II biorefineries, they are more advanced facilities that also use only one feedstock but are more flexible as they are capable of producing various end products depending on the product demand, prices and contract obligations. Finally a biorefinery Type III requires the usage of several biomass feedstocks yielding multiple end products[48], in other words, a facility that would fall in this category would be able to run in parallel at least two processes as it would have at least two raw material sources, varying from one another in composition, and in each process it would be able to harness at least one product.

This would be the case of a lignocellulosic feedstock biorefinery, whose operating methodology can be found in the following figure, using two different lignocellulosic biomasses.

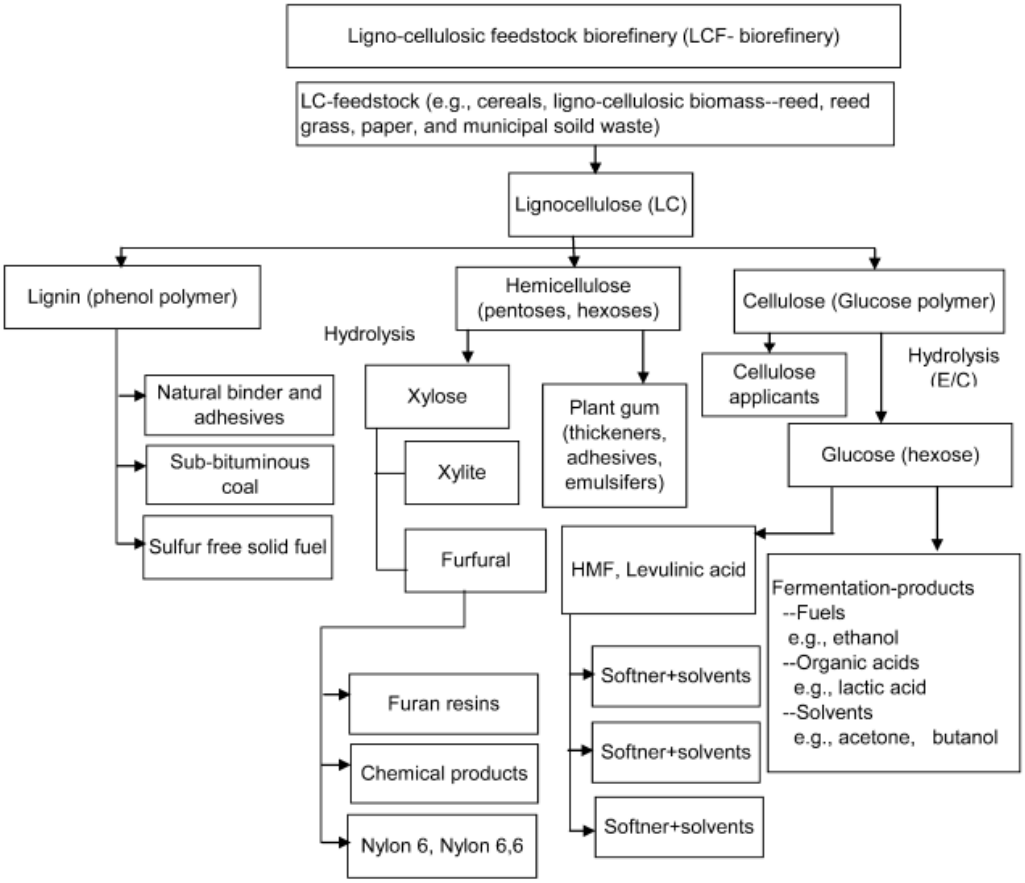


Figure 2 - Representation of lignocellulosic feedstock biorefinery process and products[48].

1.3 DESs as Alternative Solvents

The development of alternative solvents to those commonly called volatile organic solvents has been a very dynamic field, as regulations became stricter. The solvents market represents one of the largest markets in the chemical industry since, until recently, it used large quantities of cheap solvents that typically were not recycled. Supercritical CO_2, ethylene glycols, fluorinated compounds, and more recently ionic liquids (ILs) are some examples of the variety of fluids that has been recently developed to allow the implementation of more

sustainable processes. The slow development of ILs since 1943[50], offered major breakthroughs in the areas of chemistry and solvents design[51] as they allow for a multitude of combinations that in turn offer a higher process flexibility and higher selectivity. ILs have been proven to occur naturally in the wild, such as for example, when tawny crazy ants combat fire ants by spraying their venom, formic acid, mixing it with the latter's, a toxic lipophilic, alkaloid-based venom composed of 95% water insoluble 2-methyl-6-alkyl/alkenylpiperidines, and forming a protic ionic liquid[52]. These new solvents are unlike usual solvents since although in the liquid state they are composed of pairs of ions, and thus charge. ILs entered the class of green solvents due to their vanishing vapor pressure at atmospheric conditions, but their major advantage is indubitably the tailoring of their properties by the judicious choice of functional groups in the cation and anion. Although these advantages are crucial for the designing of cleaner and efficient processes, there are also problems related to their use since, for most of them, their synthesis is not green, violating green chemistry's principle of inherently safe manufacture, and alongside the fact that their production is not only expensive and time consuming, they are not readily available in industrial quantities today to implement in large-scale operations[53].

For these reasons, the development of a similar class of solvents, named deep eutectic solvents (DESs), has been getting large attention from the scientific community and also from the industry, since they have a simpler and more economical production compared to ILs while maintaining their process flexibility, high selectivity and also offsetting their major drawbacks, specifically high toxicity and nonbiodegradability[54], therefore helping in the preservation of the environment by respecting green chemistry's principles. A DES can be defined as a mixture of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), differing from ILs that only contain discreet anions[55] in a 1:1 ratio, while in DESs the ratio of HBA (typically a salt, although neutral compounds can also be used) and HBD can be any. These compounds build on the eutectic point concept to develop low temperature melting compounds, with a melting temperature lower than that of the two parent compounds. Although the most appealing concentration is that of the eutectic points, the fact there is a range of concentrations where the mixture is liquid at room temperature grants them further flexibility. Contrasting with ILs that are ionically bonded, DESs are formed by hydrogen bonding making their structure more susceptible since these bonds are energetically weaker but playing a key role in their nature as they are responsible for the depression of the melting point[56]. Also worth mentioning is the fact that they present a large deviation to ideality, as their name indicates since they are called deep because of the melting point curve presenting a particularly deep crevice at the eutectic point[57]. The following figure presents a phase diagram depicting the difference between a typical solid-liquid phase diagram for a DES and for a regular eutectic liquid, succeeded by a tabular comparison of DESs' and ILs' properties.

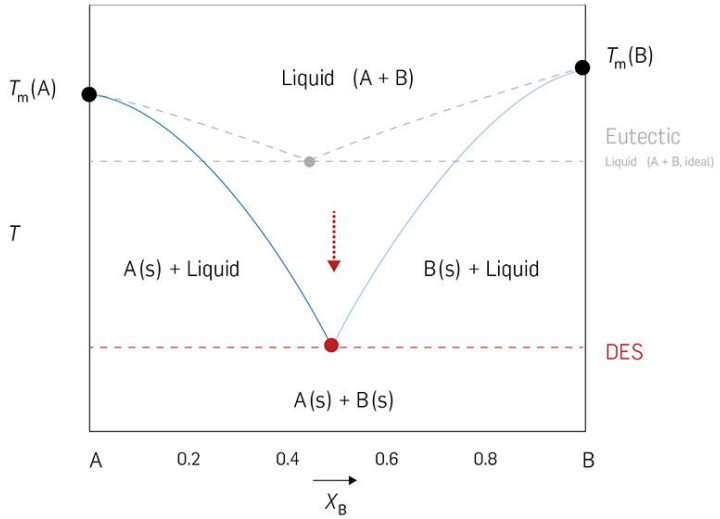


Figure 3 - Phase diagram depicting the difference in fusion temperature between an ideal eutectic mixture, in gray, and a DES, in red.

Table 1 - Comparison of the characteristics of ILs with DESs[54]**.**

Ionic Liquids	*Deep Eutectic Solvents*
Low-melting point ionic compounds	Low-melting eutectic mixture of compounds
Not always environmental friendly—can be toxic	Biodegradable and nontoxic starting materials
Solution conductivity—moderate to high	Highly conductive
Expensive—recycling is critical	Cheaper than ILs
Highly viscous	Viscosity can be lowered by mixing with suitable ionic solvents
Complex synthesis and purification required	Simple synthesis by mixing inexpensive starting components and no subsequent purification required
Moisture-sensitive ILs must be handled under dry or inert conditions	Generally not moisture-sensitive and convenient storage

DESs have only been researched since 2001, when reports presented the formation of a liquid comprised of choline chloride mixed with a metal salt (zinc chloride), each individual compound having a melting point of 302°C and 732°C respectively, at temperatures below 100°C[58]. Soon after, the same group introduced the mixture of choline chloride with urea, baptizing it as a DES, where the latter substance works as the HBD, paving the way for new research on this field for the succeeding years[59]. Mixtures of choline chloride (CC) with different carboxylic acids, such as oxalic acid (OA) or malonic acid, gave rise to a subclass of DES called

NADES, composed by natural products, namely cell metabolites[60], while other «major families» consist in the combination in pairs of a carbohydrate, like C6 sugars, an urea derivative, N,N'-dimethylurea, and a chloride salt, ammonium chloride or even in the mixture of several natural occurring acids with menthol or quaternary ammonium salts that show a hydrophobic behavior unalike the previous hydrophilic DESs[61]. The following figure presents several commonly used HBDs and HBAs.

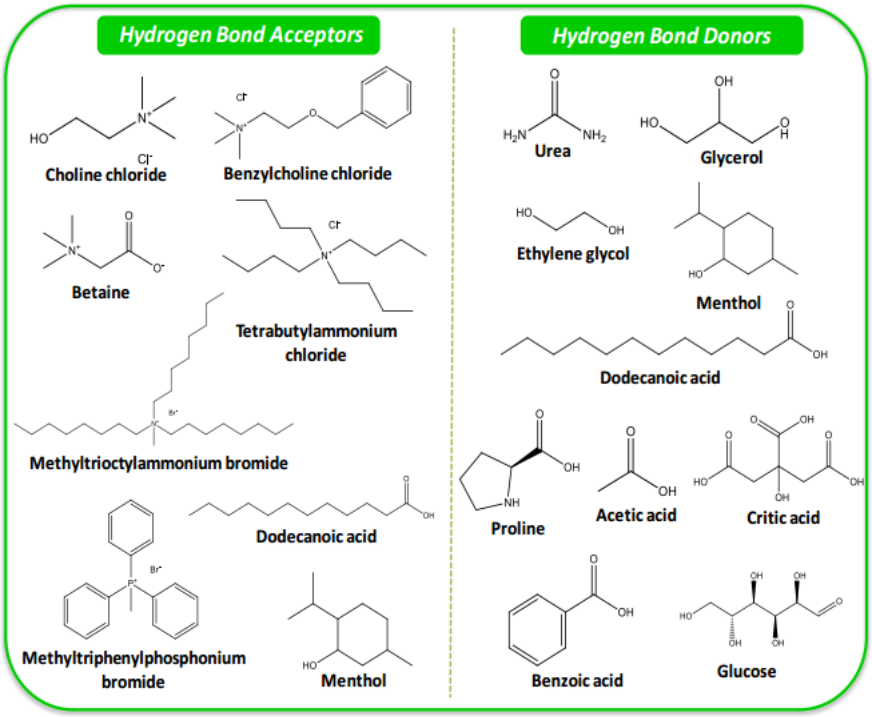


Figure 4 - Commonly used HBDs and HBAs in DESs[62].

All these variable and customizable chemicals with distinct characteristics allow for the creation of a great number of solvents with different chemical properties that can be tailored for specific procedures and have high selectivity[61]. The usage of hydrophobic DES, for instance, can be particularly effective for extraction processes in aqueous media as they have a wide range of pH environment workability since they can extract both dissociated and undissociated forms of acidic chemicals as shown in the extraction of volatile fatty acids from water, achieving greater efficiencies than conventionally used amine-based extractants[63], or even in the removal of alkali and transition metal ions, like Co^{2+}, from the same environment, managing high distribution coefficients and maximum extraction efficiency within 5s[51].

As for other DES, in general, they have also found other useful applications: the removal of post-etch residues in the cleaning of semiconductors where choline chloride mixed with urea or malonic acid proved effective, the decontamination of hazardous materials, where they showed the ability to destroy not only a wide range of halogenated hydrocarbons but also chemical warfare agents at ambient pressure and temperature with no

production of toxic by-products, the electropolishing of a large variety of metallic substrates, including stainless steels and other alloys, where operating parameters were comparable to conventional methods that involve acid-based electrolytes but a significant higher current efficiency was achieved while having the eco-friendlier advantages of not using corrosive substances, having a simplified waste management and abiding with green chemistry's principle that safer solvents and auxiliaries must be used[54]. DESs have even shown their potential to be employed in the petrochemical industry, not only in the production of hydrocarbons, specifically in fracturing where they can solubilize and remove cellulosic material utilized in subterranean drilling operations[54], but also in the production of dimethyl carbonate, a prominent gasoline and diesel additive, where they manage to breakdown the azeotrope that arises from the product's mixture with ethanol and on which is dependent the efficiency of the separation, and in turn that of the process[64].

In regards to the applications presented in this work and in the scope of the extraction and further valorization steps of rice straw, the choice of operating with DESs is therefore crucial to turning the tide on chemical engineering's record of substituting hazardous substances and as ensuring the sustainable application of these novel solvents is also a key question, the choice of using choline chloride (CC) based-DESs, although there are many other halides and options to choose from, is reasonable: its low cost and biodegradability are characteristics of the utmost importance alongside the fact that it is produced in large scale and used as a nutrient in chicken feed[61] thus being readily available in industrial quantities and following green chemistry's principle of design for safer chemicals since the cation, choline, is an essential nutrient with wide-ranging roles that vary from being a precursor in a neurotransmitter's synthesis to being a part of cell membrane signaling mechanisms and even playing a role in metabolic functions such as in the methyl-group metabolism[65] while the anion, chloride, is an inorganic halogen that is commonly found within the extracellular fluid compartment, comprising blood and plasma[66] and is responsible for osmotic pressure, acid-base balance and for the production of gastric acid among many other functions in complex living bodies[67] while also being present in many natural sources like sea water.

Alongside the employment of CC based-DESs, the utilization of octanoic acid (C8) based-DESs was envisaged as well. This carboxylic acid is one of the omega-3 polyunsaturated acids and is essential for the acyl modification of ghrelin, known as the «hunger hormone», that is vital for biological activity[68]. It is naturally occurring and can be found in the milk of several mammals as well as in coconut oil and palm kernel oil[69], being characterized by low toxicity and biodegradability that are essential for the developing of a new and clean green process; it is also widely used as an antimicrobial pesticide in a plethora of infrastructures, like commercial food handling establishments and health care facilities[70] which attests for its large-scale availability and low price. All these reasons make CC and C8 appropriate compounds for the purpose of this work.

To conclude this section it must be added that the literature revealed that no melting point for some DESs could be found and that instead they displayed a glass transition temperature, hence the sometimes attributed name «low transition temperature mixtures» for these room-temperature liquids; much like the other DES's

properties, the transition temperature depends on a wide variety of elements making the building principles hard to generalize[71]. The intrinsic solvent specific nature of hydrogen bonds is a major obstacle as they present different contact distances and binding energies that do not depend only on the donor/acceptor pair[72], unlike normal chemical bonds such as covalent bonds that can be characterized by their energy and distance and depending only on the nature of the involved atoms and their electron configuration. The use of the pK_A slide rule that involves the appropriate selection of hydrogen bonding counterparts, in a way to promote the formation of the DES and not that of an IL was considered when selecting the employed DESs; one such example is the combination of lactic acid with CC giving rise to the formation of a DES and not the corresponding IL, this one being choline lactate[71]. It should be added that for the latter to be formed, either a stronger base, with a higher pK_A, or a stronger acid need to be matched with the hydrogen bond donor or acceptor, respectively. This semiempirical rule is a pictorial summary of pK_A values, found in the literature, from diverse chemicals and organized together in order of chemical functionality for predicting the nature of the hydrogen bond; it allows for a better pairing of compounds to fit our needs as, simply put, it allows for the evaluation of the ΔpK_A and this in turn can tell us if the bond will be charged, neutral or centered as well as giving us insight on strength[72]. The fine tuning we can achieve through proton affinity/ pK_A equalization plays an important role in the formation of the DES by being responsible for the strengthening of the hydrogen bond.

1.4 Rice Straw Valorization: A Literature's Study

1.4.1 The Choice of Lignocellulosic Biomass: Rice Straw

Now as for the chosen biomass feedstock of our biorefining process, geographical and availability factors motivated the choice of using a sub-product of the rice production as our resource. Although it has more prominence in Asian countries[73], nowadays, 80% of rice production in Europe takes place in Spain and Italy with a further 12% coming from Greece and Portugal, amounting to a total of 2,85 million tons of paddy rice annually produced by these four countries, wherein this last one was of the utmost importance; after the Arabs introduced rice in the Iberian peninsula around the 8^{th} century, it spread from there to the rest of the continent, namely to Italy, and to the rest of the world with the opening of the route to the Indies in the 15^{th} century[74]. Since then, Portugal's role in worldwide rice distribution has diminished but nonetheless its rice industry remains relevant in the European context as it is the fourth biggest rice producer in Europe[75]. The following table compares several indicators to show how the production of rice in Portugal weighs up with that of the two biggest rice producers in Europe.

Table 2 - A comparison of Portugal's rice industry dimension with those of other major European rice producers in 2013[76][77][78].

Country	Italy	Spain	Portugal
Area (km^2)	302073	505944	92226
Population (millions of inhabitants)	59.69	46.73	10.49
Rice production area (ha)	212500	113200	31200
Yield (ton/ha)	6.30	7.52	5.39
Production (millions tons of paddy rice)	1.339	0.851	0.168
Rice production area / Area (ha/ km^2)	0.703	0.224	0.338
Rice production area per capita (ha/ millions of inhabitants)	3560	2422	2974
Production / Area (tons of paddy rice/ km^2)	4.433	1.682	1.822
Production per capita (millions tons of paddy rice/ millions of inhabitants)	0.022	0.018	0.016

Although the area and yield of Portuguese paddy rice fields are lower than Spanish and Italian ones, leading consequently to a lower annual production, it is still interesting to note that, proportionally, the area occupied by rice production as well as the rice production per capita and the production per area ratio in Portugal are higher than in Spain, the second biggest rice producer in Europe, thus suggesting the notorious importance of rice production in Portugal. And yet, the top European rice producers are only a speck in global paddy rice production as EU-28's production only amounts to 0,4% of worldwide rice production while that of Asian countries accounts for 90%[75]. Nevertheless, this only shows the potential of developing a valorizing process for a sub-product in rice production, more precisely, from where rice develops. The resource we will be using in this work is rice straw.

While not making use of the whole-crop potential of rice, a cereal with worldwide importance as a quarter of the world's population have it present in their diet, and its associated components like husks and bran, with the latter being rich in pharmaceutical, nutraceutical and cosmoceutical potential[79][80] and the husk being an interesting source of lignin and silica[81], rice straw is one of the most abundant available lignocellulosic biomass residues with a worldwide annual production of over 950 million tons and whereas most of it is used to feed cattle, another large portion is burnt across agricultural fields with little usefulness apart from adversely affecting nature's already susceptible fate[73] creating an urgent need for its valorization. It is also important to remark that it is estimated that this agricultural residue can potentially produce around 205 billion liters of bioethanol annually, making it the largest amount from a single biomass feedstock[73] and as it is a by-product in the production of rice just like the mentioned associated substances, rice straw should also contain added-value compounds such as antioxidants that would include vitamin E and particularly gamma-oryzanol (OZ) that will be our HVLV product and on which we will center our attention.

It is important to notice that although OZ is conventionally extracted from rice bran, rice straw was chosen due to two primary reasons; the urgent need of finding further valorization purposes for this residue is paramount to deter additional environmental pollution arising from its wasteful combustion in agricultural fields and the fact that this project wanted to prioritize the employment of resources that cannot be considered as consumable human foods. Rice bran, besides being a source of OZ, can have another important use, namely in the food industry where its nutritive value resulting from the presence of essential micronutrients like iron and B vitamins, among others, make it a serious candidate for helping the global war against famine and turning the tide on malnutrition[82].

Moreover, having selected the process's biomass, it must be stated that several other candidates were contemplated; a summary of the studied literature can be found in annex, in appendix A, where the use of other biomasses is sought for in reason of their valuable extractives too.

Now, with respect to the chosen resource, its average composition is presented in the following table.

Table 3 - Comparison of average rice straw compositions.

	Report[83]	Near infrared reflectance spectroscopy[84]	Studied literature[85]	Studied literature[73]
Cellulose (%)	[30 – 45]	[30.3 – 38.2]	34	32-47
Hemicellulose (%)	[20 – 25]	[19.8 – 31.6]	28	19-27
Lignin (%)	[15 – 20]	[7.2 – 12.8]	18	5-24
Ash (%)	[18 – 20]	[7.8 – 15.6]	7	-
Extractives (%)	-	-	13	-

It can be seen that the different sources employing different analytical methods to determine the rice straw's contents lead to similar conclusions. This was expected since the lignocellulosic biomass's composition can showcase a degree of compositional variability due to its inherent nature but these values oscillate within a set range. One of the most prominent analytical protocols was made by the National Renewable Energy Laboratory (NREL). It consists on a first step to prepare the biomass samples for compositional analysis[86], a second stage that serves to determine the extractive's content in the biomass[87] followed by the application of the «Standard Biomass Analytical Methods» protocol to finally determine the composition of the samples[88]. It is also necessary to simultaneously run a procedure to analyze the total solids content in the biomass during this final determination in order to obtain more precise results[89].

This procedure has been widely used for the analysis of lignocellulosic matter but this thesis preferentially envisaged the use of the more pragmatic Van Soest method on which the NREL's procedure is based. The Van Soest method similarly consists in a two-step acid hydrolysis. These steps involve the use of highly

concentrated sulfuric acid solutions to firstly, dissociate the holocellulose from the lignin whilst allowing the later dissolution and removal of the hemicellulose from the holocellulose fraction and secondly, remove the cellulose. This two-acid treatment stage is preceded by a digestion with a neutral detergent at 140^{o}C to dissociate the ash from the fiber fraction where the lignocellulosic contents are found. Finally, the Van Soest method includes a last calcination step where the lignin and ash are separated at 500^{o}C[90].

1.4.2 Hydrophilic DES Treatment: Fractionation of Rice Straw Biomass

In order to grasp the mechanisms and techniques that are used in this field of science, several articles were studied to get a better understanding as this work continued the research done in «**Carbohydrates-based deep eutectic solvents: Thermophysical properties and rice straw dissolution**»[91], where several DESs were tested for their rice straw dissolution potential and prepared by mixing three different HBAs with five distinct carbohydrates that served as HBDs. The results were interesting for not only understanding the protocols and equipment used in the characterization of the DES but also for designing the start of this thesis using the same preparation method of the compounds that involved mixing the two species, the HBA and HBD, in a certain molar ratio and then stirring them at 363.15 K until an homogeneous liquid phase was formed. This is commonly described as the heating method. After characterization of the formed DES by FTIR and RMN to confirm their molar ratio, the rice straw dissolution capabilities of the fifteen chemicals were tested in this article by mixing 10 mg of rice straw, previously grinded in a coffee mill to maximize mass transfer in the assay, with 2 g of the chosen DES 10 wt% aqueous solution, as the carbohydrate-based DESs are very viscous, having viscosities that range from 1000 to 12000 mPa.s[92]. Since the dissolution of rice straw in this system greatly increases the viscosity, water was added to make up for 10% of the solution's weight and reduce the mass transfer problems created by the high viscosity. It is important to mention that previous studies[93][94] observed the effect of the addition of water on the hydrogen bonds responsible for the DES formation and concluded that although they weaken with the increase of the water fraction until they disappear at about 50 v%, at 10 v% no such effect was observed. Once the rice straw is added to the DES, the assay is placed in an oil bath at 393.15K for at least 24 hrs with magnetic stirring of 400 rpm until no trace of biomass could be seen inside the flask and successive additions of 10 mg of rice straw, always spaced by at least 24 hrs, were made until the solution became turbid at which point the solubility was taken as the total biomass mass added before the turbidity was attained. This methodology, known as the cloud point method, inspired the first solubility trials of this thesis and the same procedure was used to assess this parameter, furthermore the best performing DES was chosen as a starting point to replicate the results in the article and to validate the effectiveness of the methodology. The following figure shows the results obtained for all the tested DESs in the article and where [Ch]Cl stands for CC.

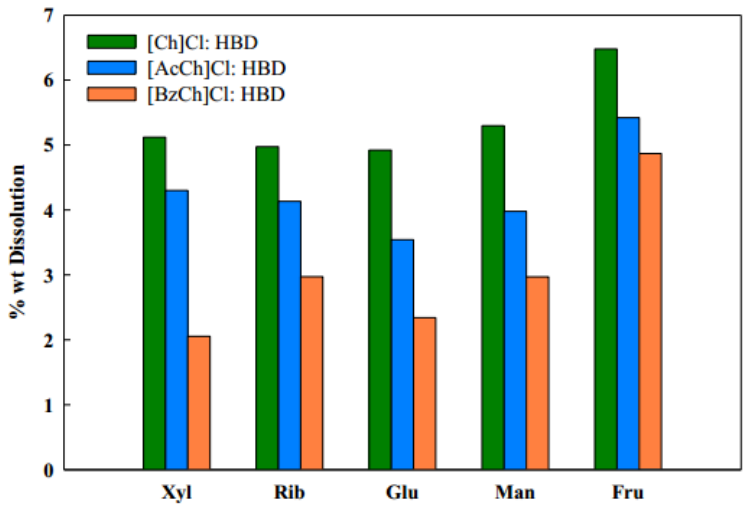


Figure 5 - Solubility of rice straw in the 15 tested DESs with a water content of 10 wt%[91].

It can be observed that the higher dissolution rates were obtained when CC was used. However, the choice of carbohydrate seems to have more impact on the results since for all the HBA, higher dissolution rates were obtained when using D-fructose-based DESs, with the highest solubility value of rice straw of 6.5 mg/g DES attained for the CC and fructose (FRU) mixture. Although the dissolution mechanism is not detailed, we can conclude that the holocelullose, which is both cellulose and hemicellulose, in the rice straw interacts with hydroxyl groups of the carbohydrates, forming hydrogen bonds. Another important parameter is the DES viscosity since the least viscous DES has the highest solubility. The authors also state that as the viscosity of the system increases, the dissolution rate slows down considerably, and it is therefore likely that the true solubility is higher than those presented in the above figure.

Alongside this article, the study of «**Deep Eutectic Solvent Aqueous Solutions as Efficient Media for the Solubilization of Hardwood Xylans**»[95] was also relevant because rice straw's hemicellulose is composed of a xylan backbone[96][97][98], making it useful for the selection of DESs for the first solubility trials and introducing the idea of separating the fractionation and the extraction steps. Furthermore, as mentioned before, the increasing water content leads to the weakening of the hydrogen bonds in the solvent culminating in their disappearance at around 50 v% and causing the DES's structure to be effectively broken down by the solvation effect of water[93][94]. The authors observed a synergetic effect that arises from the presence of both components, even if dissociated, and it therefore explains the higher extraction rates achieved with solutions through hydrotropic mechanisms, which depend on the minimum hydrotropic concentration, which is the critical concentration when hydrotropes start to aggregate[99] and the hydrotrope's ability to be a structure-breaker or a structure-maker[100]. We can indeed hypothesize that solvation spheres composed by the DES's separated compounds can occur and facilitate the dissolution process as their ability to form micelles like structures and reduce the electrostatic repulsion between head groups[101] is another mechanistic factor.

In the case of urea, a solutropic agent, it affects the solvent's nature by changing its ability to participate in structure formation by the intermediary of intermolecular hydrogen bonding[102]. Furthermore, it should also be added that the addition of water could also promote the biomass's hydrolysis by partaking in the depolymerization reaction and thus providing a higher solubility for polysaccharides[73]. However it was suggested as well that the DESs are likely to act as solvents or catalysts/ co-catalysts in the hydrolyzation of the biomass due to their acidic character achieving the loss of cellulosic crystallinity and easing the posterior enzyme's work by facilitating the accessibility to previously sterically hindered regions[71]; the increase in the water content could therefore also weaken the strength of the DES due to the pH increase of the solution thus lowering its acidic character and therefore reducing the efficiency of this treatment[73].

In what concerns the choice of DESs for this thesis's first solubility trials, it must be said that although the mixture comprised of CC and acetic acid (AA) was not the most effective one in literature, only managing a maximum of 62 mg of xylan/g of DES aqueous solution while the solvent formed by CC and urea (U) managed the extraction of 304 mg of xylan/g of solvent, it was chosen because of the mechanism by which this DES acts, not discarding the posterior usage of the CC and U DES due to its higher efficiency nonetheless. While the previously chosen FRU-based DES will interact mainly through hydrogen bonding with the rice straw components, this AA-based DES will act by another mechanistic role and cause a certain degree of acidic digestion of the biomass, as the DES's pH is between 1 to 3. Moreover, the fact that the AA-based DES is slightly more hydrophobic than the two other mentioned DESs, due to the presence of the small aliphatic chain, may also be helpful.

Similarly to the previous article, in the study of «**Deep eutectic solvents' ability to solubilize lignin, cellulose, and hemicellulose; thermal stability; and density**»[57], xylan beechwood was also used, along with medium fibrous cellulose and alkali lignin for better understanding the dissolution capabilities of the DESs. This paper presents the reason behind the need of a better delignification, also sought for in this depolymerization process, being the fact that lignin plays an important role in the lignocellulosic biomass's structure as the carbohydrate polymers composing holocellulose are tightly bound to it. This robustness, also called recalcitrance, is caused by the crosslinking between the polysaccharides with the lignin through ester and ether links[103], and acts as a barrier for getting a better glucose yield from the enzymatic hydrolysis of the holocellulose, which is the end goal of the presented scientific research, as it physically hinders the access of the enzymes to the polymeric glucose. This depolymerization step is common in the processing of lignocellulosic biomass and many of the studied articles include it as they aim to maximize the glucose yield from the posterior enzymatic hydrolysis; it is beneficial for producing saccharides fit for the manufacture of biofuels like bioethanol or even other byproducts in a biorefinery. Nonetheless, in the scope of this thesis, this digestion would rather be an additional added value process in the downstream and therefore it will not be part of the experimental framework of this chemical engineering investigation.

The following paper, «**Significantly enhanced enzymatic hydrolysis of rice straw via a highperformance two-stage deep eutectic solvents synergistic pretreatment**»[104], was paramount to this thesis's development and

it provides the description for the fractionation methodology that was used due to the effective way it implemented it. This research focuses on the need of treatment to enhance the lignocellulosic biomass's conversion into fermentable sugars as its complex heteromatrix structure made of highly crystalline cellulose surrounded by hemicellulose and lignin that occupies the remaining space preventing the exposure of the polysaccharides to water, makes it a hard to process matter. It is remarked that this treatment, when done with DESs composed of CC and acid, fares well in the processing of several biomasses but when weakly basic DESs like CC and U or neutral DESs, for instance CC and glycerol, are used, we observe only moderate sugar yields comparable with saccharification efficiencies of the untreated sources; nevertheless it is interesting to note that a 50 wt% aqueous DES solution of CC and U managed a considerable xylan dissolution, reported in the previous studied investigations, which would later reflect in this saccharification step as it is a prominent saccharide in hemicellulose, suitable for producing fermentable sugars via enzymes' employment. In summary of what was previously discussed, we must state that this depolymerization/hydrolysis step promoted by the DES alongside the decrystallization of the cellulose and the delignification of the biomass provide a suitable feed to the successive enzymatic hydrolysis of the polysaccharides hence we can achieve better results with the combination of both methods as the end product has a higher cellulose content in the recovered solid fraction and a higher reducing sugar yield.

As such this studied article focused on the use of three typical DESs, these being composed of CC and U, malic acid (MA) and proline (P) and CC and oxalic acid (OA) in molar ratios of 1 to 2, 2 to 1 and 1 to 3, respectively, for the treatment of rice straw, not only combining them with posterior enzymatic action but actually combining them in the first step of the treatment; according to the specific DESs' properties, two-DES combination treatments were utilized to ensure a greater improvement in biomass fractionation, cellulose recovery and hemicellulose degradation.

In a first stage, the three DESs, created by the heating method, were used to evaluate the solubility of pure lignin, xylan and micro-crystalline cellulose, in a screening fashion, with 10 mg of sample being added to a vial with 4 g of solvent and placing it in an oil bath at 120^{o}C with stirring; once again the cloud point method was applied and the solubility was visually checked with exactly the same procedure as described before. The mixture was then separated by centrifugation in order to collect the supernatant rich in sugar, furfural and 5-hydroxymethyl furfural (HMF) and analyze it with a high performance liquid chromatography (HPLC) system; this analytical methodology inspired the one used in this thesis to determine the sugar content of the fractionation products as well.

In regards to the treatment of rice straw with the DESs, this being the crucial part in the scope of this project, the one-DES classic treatment was conducted by incubating biomass samples at a loading of 5 wt% in the DES while being stirred at 120^{o}C. Following this step that was parameterized for a specific time, hot water was added to the assay, in a not specified amount nor temperature, to avoid the precipitation of the extracted hemicellulose and to ease not only the handling of the highly viscous resultant mixture but also its separation; it was then centrifuged at 17 000 g for 15 mn. The supernatant was collected for the obtainment of the same

specified compounds as in the screening step mentioned above and the residues were washed with distilled water until the pH of their supernatant was neutral, before lyophilizing and storing them at -20°C. In the case of the two-DES combination treatments, an identical protocol was used but the residues were subjected to the second stage of DES treatment, realized in the same conditions as the first one, after the washing step, and the supernatants of each step were combined. The results of this first stage are presented in the following table.

Table 4 - Solubility of micro-crystalline cellulose (MCC), xylan and lignin in the used DESs[104].

DES	MCC			Xylan			Lignin
	Solubility (wt%)	Glucose yield (%)	HMF yield (%)	Solubility (wt%)	Xylose yield (%)	Furfural Yield (%)	Solubility (wt%)
CO	2.9	2.2	0.8	8.6	5.0	14.7	9.4
CO:5% water	3.5	7.1	1.9	12.8	19.5	38.2	10.6
CU	3.0	0	0	12.5	0.5	0	16.7
CU:5% water	3.1	0	0	15.6	0.6	0	13.6
MP	2.4	–[a]	0	1.8	–[a]	0	2.7
MP:5%water	2.6	–[a]	0	2.0	–[a]	0	9.4

[a] Cannot be determined for the peak interference by MP.

We can see that the CC and U DES, a weak base, showcased an overall excellent solubility towards all the solutes without degradation of hemicellulose which according to the authors is in agreement with other reports while the DES composed of MA and P, a weak acid DES, managed to preferentially dissolve lignin which is also in concordance with the previous studied article that showed the latter solvent's affinity to lignin. Concerning the CC and OA DES performance, its stronger acidity made it not only dissolve xylan but it actually hydrolyzed it into xylose with further degradation into a considerable amount of furfural and the same happened in the micro-crystalline cellulose's dissolution, with its depolymerization into glucose and posterior degradation into HMF. As for the effect of water addition to the solvent, we witness an overall increase in polysaccharide solubility which can be explained by its impact on the system's viscosity, lowering it and facilitating mass transfer, as mentioned before, but also by partaking in the hydrolysis mechanism and promoting it; in the case of the CC and OA mixture, we even observe a high increase in glucose and xylose yield as well as in the HMF and furfural yield due to the water's role. The addition of water also seems to increase the solubility of lignin, with the exception of the CC and U DES where the lowered basicity resulting from the presence of water can decrease its ability to interact with lignin which is an alkaline-soluble polymer. The substantial difference in the effect of water addition in the MA and P and the CC and OA DESs can also suggest that the water's role in lignin solubility may be strongly DES dependent and that different mechanistic pathways between acidic and basic solvents exist.

Further DES treatments' results are presented in the following table where AIL stands for acid-insoluble lignin, ASL stands for acid-soluble lignin and CrI is the cellulose crystallinity index that was determined by the deconvolution method.

Table 5 - Results of the treatment of rice straw with the selected DESs and of the enzymatic hydrolysis of the residues[104].

Pretreatment				Composition of residues (%)[c]				Enzymatic hydrolysis of residues			
								Digestibility (%)		Total sugar yield (%)	
Entry	DES	Residues recovery (%)	CrI	Cellulose	Xylan	AIL	ASL	Cellulose	Xylan	Glucose	Xylose
1	Untreated	100	43.8	35.1	20.6	20.8	1.79	24.8	7.9	24.8	7.9
2	MP 12 h[a]	73.9	42.2	45.0	22.3	14.1	1.58	50.0	29.9	47.4	22.8
3	CU 12 h[a]	72.4	54.3	43.7	22.1	12.5	1.38	53.6	39.0	48.4	30.4
4	MP 6 h-CU6 h[b]	60.5	50.6	54.6	18.1	9.8	1.01	56.0	37.8	52.6	20.1
5	MP:W-CU:W[b]	57.4	68.2	58.8	17.0	8.4	0.71	77.9	57.6	75.0	27.3
6	CU 6 h-MP 6 h[b]	65.3	37.7	49.8	21.4	12.7	1.58	51.5	36.4	47.7	24.8
7	CU:W-MP:W[b]	62.2	40.2	51.9	20.0	12.1	0.75	55.0	42.0	51.4	25.8
8	MP: CU 12 h[a]	76.0	56.3	41.8	24.2	16.0	1.52	33.1	12.6	29.6	11.1
9	CO 4 h[a]	58.2	48.0	52.6	1.5	22.8	0.91	83.6	93.7	72.9	4.0
10	CU 4 h[a]	74.1	50.1	43.2	24.5	13.7	1.29	52.1	31.7	47.5	28.0
11	CO 2 h-CU2 h[b]	54.9	52.0	60.7	2.8	17.2	0.60	94.5	96.1	89.8	7.3
12	CO: W-CU: W[b]	52.5	68.5	61.3	5.0	13.3	0.54	96.2	92.6	88.1	11.8
13	CU 2 h-CO 2 h[b]	52.1	51.4	63.5	3.8	23.4	0.76	76.2	79.1	71.7	7.5
14	CU:W-CO:W[b]	52.4	50.3	64.4	5.7	14.9	0.82	77.0	83.3	73.9	12.1
15	CO: CU 4 h[a]	77.4	57.0	41.6	23.3	17.2	1.51	28.9	9.0	26.6	7.9

[a] Single DES pretreatment
[b] Two-stage DESs pretreatment
[c] Determined via the NREL protocol (LAP version 2008). The results are expressed as a percentage of the residues. AIL, acid-insoluble lignin; ASL, acid-soluble lignin.

As can be seen, the wide variety of obtained residue compositions, leading to a multitude of cellulose and xylan digestibility and overall total sugar yield, show a high selectivity in the usage of DESs putting emphasis on their potential.

Single DES treatment of rice straw with MA and P and CC and U at 120^{o}C for 12 hrs are conducive to partial delignification as attested by the lowered lignin and higher cellulose and xylan contents of the residues compared to the untreated ones, with the CC and U mix presenting slightly better results because the remaining fractions of xylan, cellulose and lignin in the residue were lower while the residue recovery was also slightly lower meaning that a higher solubility of these compounds in the DES was obtained. Regarding the performance of the CC and OA DES, we observe that it achieved a drastic removal of hemicellulose while also degrading cellulose, leading to the highest xylan removal rate and to the highest loss of cellulosic content registered in the experiment, in only one third of the time of the other DESs; this is strictly due to its stronger acidic nature.

Although the two stage DES treatment manages better results, it will not be employed in this thesis since it was particularly well demonstrated in the final stage of this article that their use leads to large amounts of wastewater being produced, subsequently augmenting waste generation. It is also hypothesized by the authors of this studied paper that a reaction between the two DESs can occur thus weakening the treatment efficiency and highlighting the fact that a compromise among process efficacy and the abiding of green chemistry's principles, namely energy savings, must be met to achieve greater results.

Thus the selection of the DESs to use in this part of the work is inspired on the results of the presented literature study but it must be observed that some other DESs were added to this list due to their functional groups that can partake in the key interactions of the lignocellulosic biomass dissolution; they are all CC based-DESs that are hydrophilic as the presence of water also plays an important part in the hydrolysis of the polymeric biomass. The selected HBDs of the DESs that are going to conduct the fractionation step are presented in the next figure along with their functional groups.

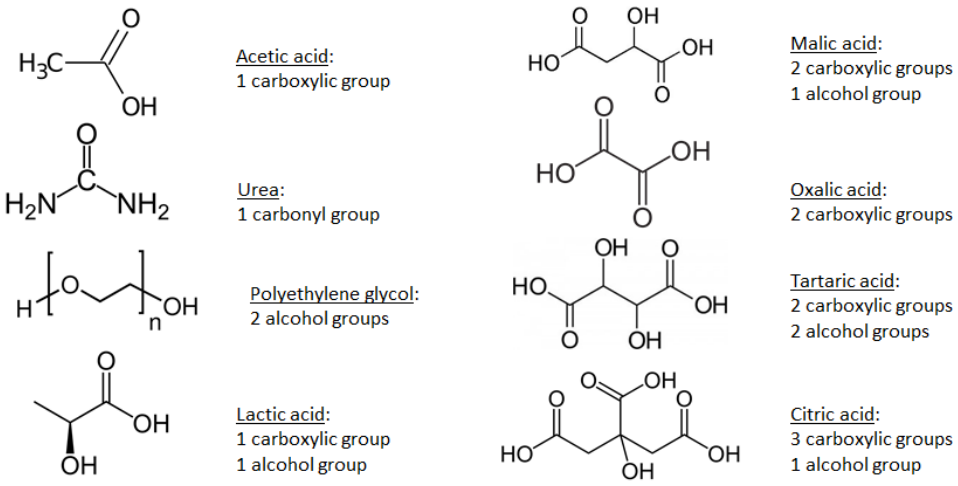


Figure 6 - The selected hydrophilic DESs' HBDs.

1.4.3 Extraction of OZ from Rice Straw

OZ was first extracted and isolated from rice bran oil in the 1950s to be used medically in Japan during the 1960s for anxiety[105] and while it was thought to be composed of one single component, it was later determined to be a group of sterol esters of ferulic acid[106]. There is still debate on exactly how many components are part of this ensemble as some studies reported that it is composed by ten different compounds, whereas others managed to identify as much as twelve ferulates from a rice bran extract[107]. Nevertheless, what is agreed upon is that it is composed of at least ten constituents[108], the three major ones being cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate[107] that together account for 80% of OZ[109] and whose chemical structures are shown in the next figure.

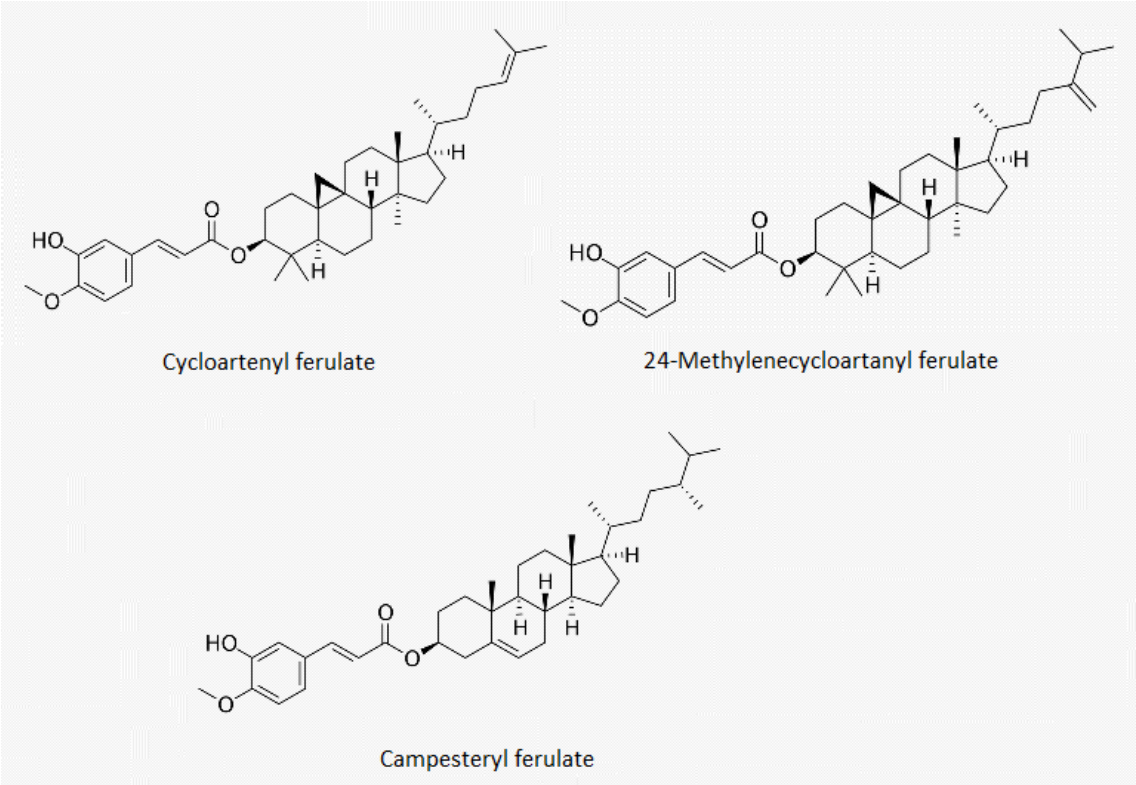


Figure 7 - The three main constituents of OZ[110][111]**.**

OZ is known to have physiological effects such as decreasing plasma cholesterol as well as platelet aggregation, hepatic cholesterol biosynthesis and cholesterol absorption at the same time that it can increase fecal bile acid excretion. It has also been used to treat nerve imbalance disorders, disorders of menopause[106] and it has been observed in laboratory animals to decrease the formation of aortic fatty streaks[107]. Besides the hypolipidemic properties already mentioned, OZ has many other biological properties and health benefits that include antioxidant, anti-inflammatory, antineoplastic, hypoallergenic, antidiabetic and antiulcerogenic[107][108]. Overall, it is a very interesting set of compounds with promising applications in the pharmaceutical, nutraceutical and cosmetic fields where it is already being sold as either a supplement[105], a drug or even used in creams and sunscreens due to its skin age resistor function derived from its UV absorbing effect[112][113][80]. It also has a high monetary value as a specialty chemical[114].

An additional note must be added concerning the choice of operating with eco-friendly DESs in this academic study. This choice is distinctly important because the extraction step on which we will focus on suffers from further restrictions, apart from those mentioned above. It is regulated by legislation with the European Union imposing solvent restrictions in this first step if the compound that is to be extracted can be classified as natural and fit for direct usage on humans[41], which is the case of OZ. While only the employment of water, (bio)ethanol and CO_2 are allowed in the processing of natural products[41], the interest in novel extractions

using green solvents is increasing making this an essential investigation as the need for new, clean and efficient biomass conversion technologies persists.

In regards to the existing research done on OZ extraction, it has been mostly performed in Japan where 7,5 annual kton of this compound are manufactured from 150 kton of rice bran[105] using organic and inorganic solvents that do not abide with green chemistry's principles, hence indirectly contributing to the environment's deterioration. Patents on its production consistently showcase the use of caustic soda and acids like hydrochloric acid or sulfuric acid to obtain an OZ-rich phase from the rice bran oil production[115][116] that is in turn produced by high temperature incubation of rice bran with solvents like hexane or caustic soda solutions, once again[117][118].

The study, in this section, of «**Gamma Oryzanol from Rice Bran Oil-A Review**»[118] will have the objective of giving an important elaboration on the conventional methods for obtaining OZ, as it describes the production of rice bran oil from rice bran and the different methods applied for the extraction of OZ from it as this group of compounds exists at a level of 1 to 2 per cent in the oil, where it is biologically useful as a natural antioxidant.

Rice bran oil is normally extracted from rice bran using food grade n-hexane but other methods like a solvent free process that consists in ohmic heating or a supercritical fluid extraction were also developed; as the common utilization of n-hexane in the production of rice bran oil has many offsetting factors in terms of health and environmental hazards, not even mentioning the processing hazard of operating with an inherently dangerous chemical, efforts to find substitutes for this process and this compound were made. Short-chain alcohols, specially ethanol and isopropanol, have been applied as alternative extraction solvents as they abide with a greater number of green chemistry's principles and have not only a reduced probability of regulation but a lower cost as well. Interesting investigations were led achieving promising results where ethanol usage was shown to yield a rice bran oil richer in tocopherols, tocotrienols and B vitamins at the same time that isopropanol utilization managed to extract rice bran oil richer in B vitamins alone; because these alcoholic solvents have a greater polarity, they are able extract more non-glyceride material from the oil[119]. Some of these researches also used a mixture of the alcohols with hexane[120] and furthermore, it must be remarked that in one of these particular studies where ethanol was used, the contents in OZ of the extracted rice bran oil were determined, leading to the conclusion that 1500 to 4000 mg of OZ can be extracted from 1 kg of fresh rice bran, depending on the conditions, and showcasing the feasibility of using ethanol in this process[121] as the achieved results are comparable with those obtained with hexane as will be later discussed; studies are still being developed for optimizing the employment of ethanol in the extraction of rice bran oil from the rice bran[122]. Yet, it is important to mention that, in certain cases, the use of isopropanol and methanol has bested the use of hexane as an extraction solvent; a report mentioning a better extraction yield with these solvents at a 1 to 60 weight/volume ratio, in other words a 1.67 wt% biomass loading, showcases that in these conditions it is favorable to use a substitute solvent over the conventional one[123] and although further quantities of solvent will be needed to achieve the same yield than conventional methods under normal

conditions, indirectly contributing to the loss of process efficiency due to economic and energetic factors, this is still a clear indicator that a substitute can indeed be found.

The other alternative oil extraction procedure that is reviewed in the studied article is the usage of supercritical fluids, namely dense and critical CO_2; the solubility of rice bran oil and some of its constituents in it was studied to validate the feasibility of using this technique in order to add further value to this biomass and other investigations were led to determine the possibility of scaling up the supercritical fluid fractionation process. The selective enrichment in sterols and lipid species of rice bran oil achieved with this method was evaluated and moreover it is reported that a total of 4.93 g of rice bran oil can be extracted from the bran with only 100 g of CO_2 at 80^{o}C and 10 000 psi pressure, drastically reducing the use of solvent in the oil extraction process.

The following table presents the OZ yield in the obtained oil from rice bran with these two mentioned alternative methods.

Table 6 - Comparison of OZ yield by solvent extraction and supercritical fluid extraction (SFE)[118].

	Solvent extraction[a]	SFE[b]	SFE[c]
Yield (mg/g of rice bran)	1.68± 0.02	5.39±0.43	1.11±0.07
Concentration (mg/g of extract)	11.8± 0.2	51.0± 5.5	674.6± 148.1

[a]Extraction with hexane/isopropanol (50:50) at 60^{0}C for 60 min
[b]Extraction under 680 atm at 50^{0}C for 25 min
[c]Extraction under 680 atm at 50^{0}C and collection between 15-20 min

It can be seen that even the highest reported OZ yield with organic solvents is four fold smaller than the one achieved with supercritical fluid extraction and where a shorter amount of processing time can actually lead to a tenfold higher concentration of OZ in the extract. However as promising as these results are, supercritical fluid extraction still faces many adversities, specially the high cost of the equipment and its operation as well as the involvement of solvents at their critical point being inherently unsafe[119]. For these reasons the employment of the solvent extraction methodology with hexane for obtaining the rice bran oil (RBO) is still prominently used.

The oil is then further processed by chemical or physical refining to meet the pretended specifications of edible grade vegetable oil and while the chemical purifying process results in better product in means of color and cloud point than the physical procedure, it causes higher refining losses that can be in the forms of wax and gum sludge or soapstock which is a byproduct of caustic refining[124]; this is troublesome as these residues are a good source of many other substances like OZ, tocopherols, vitamin E, ferulic and phytic acid, sugars and even fatty substances like lecithin or wax that go unused and end up as waste, lowering the process efficiency.

Once the crude RBO has been extracted from the bran, the aforementioned illustrated refining takes place using different processing techniques, either physical or chemical, and it is interesting to notice that their employment leads to a major difference on the retention or availability of OZ resulting from the invasive

properties of the different procedures; in the physical refining of RBO where no alkali treatment is applied, the article reports a 1.1 to 1.74% content of OZ in the extracted oil while for the one submitted to chemical refining, involving this particular treatment, the content is much lower at 0.19 to 0.2%. These results showcase the appreciable loss of the compound during the alkali refining step that conduces to the removal of approximately 94% of the total OZ present in the original crude oil. Nonetheless, it is this same efficiency that makes the alkali treatment such an important process for extracting the OZ from the RBO, thus explaining the common use of caustic soda for obtaining OZ-rich phases and products.

An illustrative procedure to refine the RBO, using not only strong alkaline solutions but also strong acidic ones, for obtaining OZ, consists in stirring the RBO with a 90% NaOH aqueous solution and storing it at room temperature for one day before conducting the rest of the process; afterwards it is acidified to pH 4 with sulfuric acid, heated at 80^{o}C and followed by a NaCl addition to give an oily layer that is washed with an aqueous NaCl solution. Subsequently, methanol is added to extract as much water as possible from the mixture before cooling it for 2 hrs at 0^{o}C to precipitate the waxy components and filtering them to allow for the filtrate to be mixed with a sulfuric acid/methanol solution. After 24 hrs at 30-40^{o}C with periodical stirring the methanol was evaporated and 2 wt% of acid clay was added before heating the mixture at 100^{o}C under vacuum for 30 min and filtering it once again to follow it with a distillation of the filtrate at 2 mm pressure. N-hexane was then added in a 1 to 1 proportion with the residue's mass while stirring and another distillation was carried out before repeating this same treatment with methanol, in the same proportion. The residue was extracted 3 times with methanolic NaOH and finally the extracts were neutralized with methanolic acetic acid until the pH was 6.8-7 and stored to precipitate 3g of raw crystallized OZ from the original 1 kg of raw RBO.

In order to conclude on the OZ's extraction and analysis methodology, the next article, «**Genotype and environment effects on tocopherol, tocotrienol, and γ-oryzanol contents of Southern US rice**»[107], although employing rice bran and aiming to determine the effects of genetics versus environment on the OZ contents of rice, conducts a simple and efficient procedure to extract OZ and an equally appropriate analytical method, similar to the ones of previous studied articles, to determine the OZ concentration in the extract.

The employed extraction procedure consists in the addition of 250 mg of rice bran to a flask containing 5 mL of hexane and vortexing it for 15 s before introducing the flask in a 60^{o}C water bath for 30 min. During the incubation, the assay was vortexed twice and afterwards, the hexane layer was separated from the rice bran sample by centrifuging at 2000 g for 10 min. After separating the liquid and solid phases, an additional 5 mL of hexane were added to re-extract the rice bran residue without incubation to allow for another phase separation. Then, the two separated hexane layers were combined together before completely evaporating the solvent under nitrogen flow; 1 mL of hexane was added prior to the analysis.

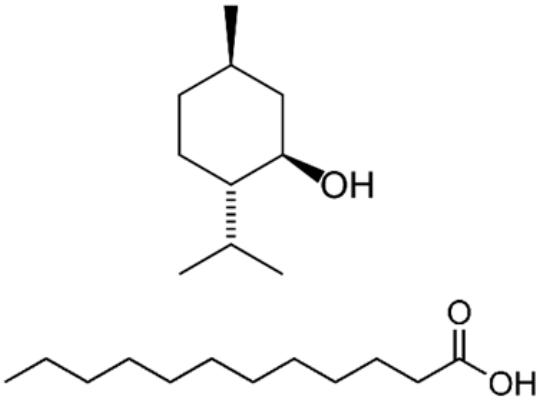
It must be remarked that this extraction method is indeed not only simple and efficient but it was also adapted from the best performing extraction procedure[125] according to another study, where several OZ extracting methods were applied and compared with one another[120]; the adapted extraction procedure will be employed in this thesis to obtain OZ from rice straw due to its efficiency and as it does not involve a

saponifying step, an important factor since it is detrimental to the recovery of OZ. The article «**Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide**»[79] also reinforced the need to adopt an extraction procedure that avoids using a saponification step as it lowers the extraction recovery. This step has been employed in most lipid extractions from plant and animal tissues as it eases the release of lipid from the sample matrix and also helps remove interfering chemicals like triglycerides and other hydrolysable material; the problem arises precisely from this last effect as saponification may hydrolyze the ester bond between triterpenoids and ferulic acid components of OZ. Furthermore it must be underlined that the used temperature in this process does not affect OZ's structural integrity as it was found to be stable at temperatures up to 120^{o}C[126][127][128].

As for the analytical method used in this studied paper, it consisted in transferring the extract phase of 1 mL of hexane solution to an HPLC sample vial and introducing it in a 25 cm x 4.6 mm diameter column of 5 µm Supelcosil LC-Si which was preceded by a 5 cm x 4.6 mm guard column packed with 40 µm pellicular silica through a 715 Ultra WISP injector powered by a 510 pump. A 486 UV detector from Waters running with Waters Millennium chromatography software was used to detect the OZ fraction in the eluate at 330 nm. The mobile phase consisted of 0.5% ethyl acetate and 0.5% acetic acid in hexane at a flow rate of 1.5 mL/min. It can be noted that the method involves a HPLC which is the conventional method for quantifying OZ in extracts, as also verified by the study of the literature[79][106][108] since it conduces to clear and efficient results; as such, it will be used in this thesis as well.

The high extraction yields obtained with this methodology, varying from 2.5 mg of OZ/g of rice bran to 6.9 mg of OZ/g of rice bran, attest for the efficiency of this process and validate the choice of these procedures.

In conclusion, it must be mentioned that as no OZ extraction from rice straw has ever been reported in the literature, moreover with a DES, the chosen combinations of C8:Menthol and C8:Dodecanoic acid were elected due to their hydrophobicity, an important factor as already discussed since it is the common denominator in all of the above studied articles wherein OZ was extracted. The following figure presents the structures of the HBDs.



Menthol:
1 alcohol group

Dodecanoic acid:
1 carboxylic group

Figure 8 - The selected hydrophobic DESs' HBDs.

1.5 Objectives

In regards to the objectives of this work, we will delve in this field of biomass processing with the goal of harnessing a HVLV product and the prospects of producing a LVHV product in order to increase the process value, while always ensuring the application of the green chemistry principles to satisfy the demands of sustainable development while reaching for the end goal of sustainability.

Therefore, in this study, OZ extractions from rice straw using DESs will be attempted following a previously reported experimental protocol for organic solvents, where a heating in shake flasks for a fixed amount of time and with periodic agitation is performed. After the compound's extraction, the flasks were subjected to centrifugation to separate the solid biomass residue from the OZ-rich extract phase that was then analyzed according to reported methods to validate the feasibility of this methodology. A separate fractionation study of the rice straw was also conducted in order to assess the potential of developing a post-treatment for the extraction step's residues, leading to a high fermentable sugar yield with the aim to produce a LVHV product to bring added value to the global process.

In this thesis, the reader can find a thorough literature introduction to sustainability and societal challenges, green chemistry and biorefineries, the motivation to select rice straw as the biomass feed stock, the selection of the DESs along with the description of the treatments used to process several lignocellulosic biomasses and the conventional routes for obtaining OZ that influenced the extraction step's protocol which was designed to achieve the production of a HVLV product. This is followed by an experimental section where the list of the materials and equipment utilized in this work are described as well as the experimental methods dictating the conduction of the experiments. Finally, the results are presented and discussed to allow for a conclusion to be made on this thesis's final objective of answering the succeeding problematics:

-Can an efficient process consisting in the usage of DESs to extract OZ from rice straw be developed? If so, how does it compare with current used technologies that are employed to valorize this lignocellulosic residue?

-Is there potential to develop an efficient treatment process using the extraction step's residue? If so, how does it compare with other fractionation protocols' results?

2 Experimental Section

2.1 Reagents

The following table presents the chemical substances used in the development of this project including the suppliers, purity level and other relevant properties.

Table 7 - List of the required reagents for the project.

Chemical Compounds and Acronyms	Supplier	Purity (%)	Molar Mass (g/mol)	Melting (MP)/boiling point (BP) (°C)
Choline chloride (CC)	Sigma-Aldrich	≥98	139,62	302-305 (MP)
D-(-)-Fructose (FRU)	Sigma-Aldrich	≥99	180,16	119-122 (MP)
Urea (U)	Sigma-Aldrich	≥99,9	60,06	132-135 (MP)
Acetic acid (AA)	Sigma-Aldrich	≥99,7	60,05	117-118 (BP)
L-(-)-Malic acid (MA) DL-Malic acid (MA)	1) Sigma-Aldrich 2) Sigma-Aldrich	97 ≥99	134,09 134,09	101-103 (MP) 131-133 (MP)
L-(+)-Lactic acid (LA)	Sigma-Aldrich	90	90,08	110 (BP)
L-(+)-Tartaric acid (TA)	Carlo Erba	≥99	150,09	-
Citric acid (CA) (monohydrated)	Pronalab	≥99,5	210,14	-
Polyethylene glycol (PEG 200)	Sigma-Aldrich	100	200	-65 (MP)
Oxalic acid (OA) (dihydrated)	1) Sigma-Aldrich 2) BDH	≥99 ≥99,5	90,03 126,07	189,5 (MP) -
Octanoic acid (C8)	Acros Organics	99	144,21	238 (BP)
DL-Menthol (M)	Sigma-Aldrich	≥95	156,27	34-36 (MP) 216 (BP)
Dodecanoic acid (C12)	Sigma-Aldrich	98	200,32	44-46 (MP) 225 (BP)
Gamma-oryzanol (OZ)	Sigma-Aldrich	100	602,89	-
n-Hexane (H)	Carlo Erba (HPLC grade)	≥99	86,18	-

It must be noted that two different oxalic and malic acids were used due to their availability in the laboratory. The dried rice straw was obtained from **C**OTARROZ, **Centro Operativo e Tecnológico do Arroz**, Salvaterra de Magos. The rice straw was cut and grinded using a regular coffee mill into particles with a size of 0.5-1 mm approximately, and then stored in a sealed plastic container that was kept under atmospheric conditions preceding its utilization.

2.2 Material and Equipment

The laboratory material as well as the small equipment used in the preparation of the DESs and in the extraction and fractionation experiments is presented in annex, in appendix B.

Moreover, a Karl-Fischer apparatus, 831 KF Coulometer from Metrohm, coupled with a 728 magnetic stirrer, from Metrohm as well, was used to determine the water content in the selected solvents. A UV-vis spectrophotometer, UV-1800, from Shimadzu was used for the preliminary analysis of the extraction step's experiments with a UVProbe v2.34 software from Shimadzu. A HPLC composed of a Phenomenex Luna Silica (2) 100 Å 250 mm x 4.6 µm column coupled with a UV detector was used to determine the OZ content in the extraction step's experiments and an HPLC apparatus composed of a CarboPac PA 10 4x250 mm column preceded by an Aminotrap and coupled with a pulsed amperometric detector was used to determine the sugar content in the liquid extract phases obtained from the fractionation step.

Apart from the listed equipment, common laboratory glasswork like beakers, pipettes and glass flasks, was used.

2.3 Methods

2.3.1 Preparation of DESs

The methodology used to prepare the DESs was based on the heating method that is previously described in the literature study section[91]. The starting materials were either weighed on a scale or measured with a pipette or a syringe if they were in solid or liquid form, respectively, and dried prior to use: CC and U were dried for at least 24 hrs in an oven at around 45^{o}C, while PEG 200 was dried for at least 24 hrs under vacuum. All the other starting materials were used without further processing.

The HBA and HBD composing the DES were then mixed in a sealed shake flask placed in an oil bath at 80^{o}C with 400 rpm magnetic stirring for at least 1 hr. Once a clear homogeneous liquid was obtained, the heat was turned off and the DES was let to cool down until it reached room temperature. Before being used, the DES's water content was measured using a Karl Fischer apparatus. In the case of the hydrophilic DESs used in the

fractioning of rice straw biomass, water was added in order to prepare the DES aqueous solution. The necessary water mass is calculated by the following equation, where w is the desired water weight fraction and the $mass_{DES\ humidity}$ was determined by the Karl Fischer apparatus:

$$mass_{water} = \frac{w}{1-w} * (mass_{DES} - mass_{DES\ humidity}) - mass_{DES\ humidity} \quad (1)$$

As for the hydrophobic DESs employed in the extraction of OZ, they were used immediately after the determination of the water content.

It must be mentioned that an investigation on the preparation of the hydrophilic DESs in an aqueous media was also conducted; in these experiments, the above described steps for the preparation of the DESs were carried out in the same manner but in reverse order, with the addition of the HBA and HBD after introducing the water in the flask, followed by the application of the same heating method.

As the DESs were all already reported and characterized in the literature, the following table presents the chosen molar ratios of the selected DESs. Typically, the eutectic composition was chosen to afford a higher liquid working range but sometimes a composition range is available where the DES is liquid at room temperature. When there is the possibility to use several possible molar ratios, the most economic and eco-friendly one was used, meaning that higher molar fractions of HBD, which are organic acids, sugars, alcohols, were privileged in comparison with HBA, which is a salt.

Table 8 - Molar ratios of the used DESs and the step where they were used.

	DES (HBA:HBD)	HBA:HBD Molar ratio	Step of the Process
1	CC:AA	1:2[95]	Fractionation experiments
2	CC:U	1:2[95]	Fractionation experiments
3	CC:PEG 200	1:4[129]	Fractionation experiments
4	CC:LA	1:1[130]	Fractionation experiments
5	CC:MA	1:1[60]	Fractionation experiments
6	CC:OA	2:1[104]	Fractionation experiments
7	CC:TA	2:1[130]	Fractionation experiments
8	CC:CA	2:1[60]	Fractionation experiments
9	CC:FRU	1:1[91]	Fractionation experiments
10	C8:M	1:1[131]	Extraction experiments
11	C8:C12	3:1[132]	Extraction experiments

2.3.2 Extraction of OZ from Rice Straw

Solubility tests were carried out to conclude if the usage of the two selected hydrophobic DESs in the extraction step was appropriate; 1,5 mg of OZ were added to approximately 2 g of DES in a shake flask that was then vortexed for at least 30s. The shake flasks were left to equilibrate at room temperature with vigorous stirring, for at least 24 hrs before conclusions were drawn.

The extraction protocol of OZ from rice straw was carried out according to Bergman et al.[107], with slight modifications. The procedure for the extraction essays with hexane consisted in mixing 250 mg of rice straw with 5 mL of hexane in a shake flask, vortexing for 15s and incubating the mixture in an oil bath at 60^{o}C for 30 min while vortexing for 15s at the 10 and 20 min mark. Subsequently, the hexane layer was separated from the rice straw sample by centrifuging for 20 min at 6000 rpm and another 5 mL of hexane were added to re-extract the residual biomass sample without incubation but with a single vortexing of 15s. Another phase separation was conducted under the same conditions and finally the two separate hexane layers resulting from the two extraction steps were mixed, filtered with a syringe filter to ensure that no suspended particles were present and then completely evaporated under nitrogen flow. A posterior addition of 1 mL of hexane was carried out before the HPLC analysis of the assay. OZ standards were acquired from Sigma-Aldrich.

The same protocol was applied when the two hydrophobic DESs were used, with two exceptions: instead of using 5 mL of hexane, the corresponding mass of DES was used and the final evaporation step of the extract phase's solvent was not conducted and the samples were analyzed after the filtration. The residual biomass obtained from these experiments with hydrophobic DESs was dried in an oven at 45^{o}C for at least 72 hrs to assess if the solvent could be evaporated.

Additionally, blanks of the extraction experiments were made with each solvent to provide for a control and a baseline in the HPLC analyses; they followed the same procedure than the other respective assays but in the absence of rice straw.

2.3.3 Hydrophilic DES Treatment: Fractionation of Rice Straw Biomass

In order to determine what would be the optimal DES concentration in the DES aqueous solution for achieving the highest rice straw solubility and in theory the best depolymerization yield of the lignocellulosic biomass sample, as well as to validate the results obtained by Florindo et al.[91], previously described in the literature review section, a preliminary rice straw dissolution test was conducted where two CC-based-DESs were representatively selected. Different water fractions were added to shake flasks containing 4 g of DES composed of CC:FRU or CC:AA to create aqueous DESs solutions with a water content ranging from 10 wt% to 50 wt%. Following this step, the cloud point method previously described in the literature review study was applied to determine the solubility of the lignocellulosic biomass in the different solvents. The tests were conducted at the temperatures of 60^{o}C and 90^{o}C. Small increments of 1 and 4 mg of rice straw at 60 and 90^{o}C, respectively,

were added until turbidity was spotted, at which point the sample was left to equilibrate for at least 24 hrs. After this period of time, if turbidity had not disappeared, the hitherto added mass was used to calculate the solubility and if otherwise, another small biomass increment was made until turbidity or undissolved particles were noticed, triggering the repeat of the equilibration process.

Once the water fraction leading to the highest solubility results was determined, a preliminary screening of the DESs' rice straw depolymerization potential was carried out, using the same exact methodology described above with the exception that the test was conducted at 25 and 50^{o}C with biomass increments of 0.5 mg of rice straw in 1 g of the selected DESs. It must be noticed that the assays at 50^{o}C were performed in 1.5 mL Eppendorf containers, heated and stirred in the Thermomixer.

At last, the best performing DESs were selected and employed to achieve the fractionation goal presented in the literature[104]. The one-DES treatment was chosen over the two-DES treatment not only for pragmatical reasons but also to avoid the production of large quantities of wastewater because the efficiency of the two-DES process is highly reliable on a thorough washing of the residues before the employment of the second DES to ensure that no residual DES is left from the first step. As the used hydrophilic DESs in this step are highly viscous, large quantities of wastewater would be necessary to completely remove them. As such, the fractionation procedure consisted in incubating rice straw samples at a loading of 5 wt% in approximately 6 g of DES in a shake flask that was heated at 120^{o}C for 18 hrs with a magnetic stirring of 400 rpm. The time was selected as the aforementioned study reported better results in terms of biomass solubility in the DES and of cellulose concentration of the residues for longer processing times in single-DES treatments. At the end of this incubation, 20 mL of hot (68^{o}C) water were added to the hot mixture, a centrifugation at 4500 rpm for 30 min was carried out and both the DES-rich extract phase and the wet solid biomass residue were left, separately, to equilibrate for at least 48 hrs in sealed flasks under atmospheric conditions. Afterwards, the extract and the solid phase were filtered by Büchner filtration, in this order, using the same pre-weighed paper filter and washed with Milli-Q water to make up a 200 mL filtrate. The paper filters were left to dry for 24 hrs in an oven at around 45^{o}C before weighing them again to exactly determine the amount of collected undissolved biomass residue and the extract phases were stored in a refrigerator at -2^{o}C prior to their analysis. Furthermore, blanks of the fractionation experiments were made with each DES to provide for a control and a baseline in the HPLC analyses, following the same procedure than the other assays but in the absence of rice straw.

Finally, an optimization of this fractionation step was conducted to determine what would be the optimal time in order to achieve the most promising results while reducing the processual energy expenditure. Therefore the duration of the incubation, that was previously 18 hrs long, was reduced to 4 and 8 hrs. Apart from this, the optimization experiments were performed under the same conditions as aforementioned.

2.3.4 Analytic Procedure

The OZ content determination in the DES-rich extract phase obtained during the extraction procedure was preliminarily analyzed to validate the need of an HPLC system to determine this information. A UV-1800 Spectrophotometer was used to obtain the UV-vis spectrum of the extraction matrix, using wavelengths ranging from 190 to 500 nm at a medium scan speed and auto sampling intervals, while the data was processed with the software UVProbe v2.34. The solubility tests of OZ in the selected DESs were also submitted to the same analysis in order to validate the location and the characteristic shape of the OZ's peak in these solvents. Literature shows that OZ's peak is located around 330 nm[107][79] as this is the characteristic wavelength of ferulic acid's UV peak, one of the OZ's compounds' architecting blocks[111]. Following this, the extract phases were analyzed through a normal-phase HPLC, with a system consisting of a Phenomenex Luna Silica (2) 100 Å 250 mm x 4.6 µm column coupled with a UV detector set for a wavelength of 330 nm. The mobile phase was composed of hexane, isopropanol, ethyl acetate and acetic acid in a 94:5:0.5:0.5 volumetric proportion and 20 µL were injected at a flow rate of 1.5 mL/min. The analysis was conducted at 30^{o}C and the standards were prepared using the acquired OZ in hexane at different concentrations in order to obtain a calibration curve. A posterior addition of the acquired OZ in the extraction assays was also carried out to conduct further HPLC analyses, under the same conditions, and conclude on the effectiveness of the extraction procedure.

The cloud point method was used to determine the rice straw's solubility in all of the conducted solubility analyses and this variable was visually determined according to consistent criteria consisting in the presence of either turbidity or undissolved particles. The content of dissolved sugars in the residual DES aqueous solution obtained in the fractionation procedure was also analyzed by an ion-exchange HPLC, using a CarboPac PA 10 4x250mm column preceded by an Aminotrap and coupled with a pulsed amperometric detector. The mobile phase was composed of an 18 mM NaOH solution and 10 µL were injected at a flow rate of 1 mL/min. The analysis was conducted at 25^{o}C and the standards were prepared using ultrapure water with the addition of a known quantity of the following monomeric sugars: xylitol, arabitol, fructose, arabinose, galactose, glucose and xylose. The standards, differing in concentration, allowed for a calibration curve to be plotted.

3 Results & Discussion

3.1 Extraction of OZ from Rice Straw

This thesis's section presents the development of a process aiming to produce a HVLV product from rice straw, namely an extract rich in OZ obtained by using hydrophobic DESs and thus contributing to the biomass's valorization; this part consists in an assessment of the OZ solubilizing ability of the selected DESs, a preliminary analysis of these solubility tests and of the OZ extraction assays, a HPLC analysis of the OZ extraction assays and finally an analysis of these DESs' recyclability.

3.1.1 OZ Solubility Tests in Hydrophobic DESs

The following pictures present the results of OZ solubility tests carried out in C8:C12 DES on the left and in the C8:M DES on the right.

Figure 9 - OZ solubility tests in hydrophobic DES: C8:C12 DES on the left and C8:M DES on the right.

According to the studied literature, an average of 4 to 4.5 mg of OZ can be extracted from 1 g of rice bran by employing the extraction methodology of this thesis[107]; assuming that the used DESs can extract as much OZ as hexane and rice straw could contain as much OZ as rice bran, since it not only composes the rice plant but most importantly partakes in the growth of rice grains from where the rice bran is obtained, OZ was added in excess to the two tested DESs, C8:M and C8:C12, to assess if the solvents would be able to solubilize it and therefore be eligible to carry out the extraction. Therefore a value of 1,5 mg of OZ was added to 2 g of each DES to reach a concentration of 750 ppm, over fourfold higher than the hypothetic one that would be obtained in the real extractions where 250 mg of rice straw are going to be added to 6,5 g of DES, achieving a concentration of approximately 163 ppm taking into account the premade assumptions on the OZ concentration in rice straw (4.25 mg of OZ/g of rice straw) and on the extraction capabilities of the DESs.

As can be seen from the above figure, both the DESs, C8:C12 on the left and C8:M on the right, managed to completely dissolve the added OZ, with no trace of any undissolved particle or any turbidity, thus making their application in the extraction procedure appropriate.

Following this step, the effect of the chosen solvents on the OZ detection by UV-vis spectrophotometry was studied by analyzing the solubility tests with a UV-1800 spectrophotometer. It must be mentioned that the solubility tests were diluted by a factor of twenty to obtain unsaturated spectra of the DES/OZ matrix. Furthermore, the assays obtained from the extraction procedure of OZ from rice straw were also analyzed by this same method.

3.1.2 Preliminary Analyses of UV-vis Spectra

The solubility tests' UV-vis spectra are hereby presented, followed by those of the extraction matrixes where the assays relative to the C8:C12 DES are on the left and those relative to the C8:M DES are on the right.

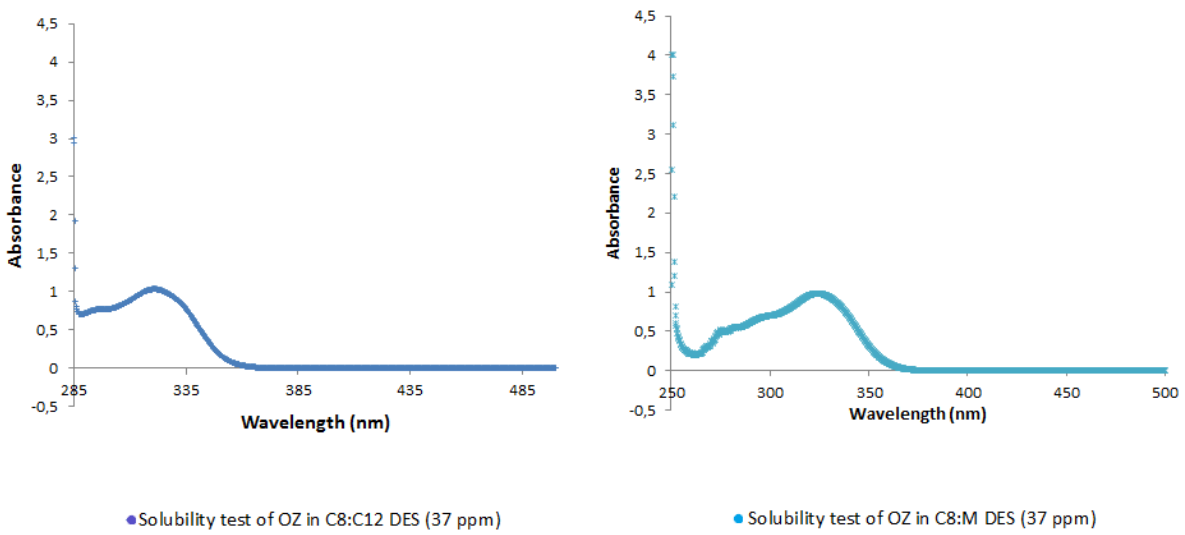


Figure 10 - Spectra of the OZ solubility tests in the two selected DESs.

After proceeding with the dilution of the solubility tests in order to obtain solutions with a OZ concentration of 37 ppm, leading to unsaturated spectra, it was observed that in both cases the absorption bands present a maximum at a wavelength of 330 nm; this is the wavelength corresponding to ferulic acid[111] that is one of the architecting blocks of the compounds present in OZ.

This analysis served to better understand the characteristic absorption bands of OZ in these solvents while later allowing to infer on the successfulness of the OZ extraction in the assays. Additionally the obtained absorption bands in the spectra are identical to those presented in the literature for cycloartenyl ferulate, one of the major OZ's compounds[133][134]; this is explained by the fact that the OZ standard that was used in this work is composed of cycloartenyl ferulate, also known as OZ A[134].

It must also be stated that there is some signal interference of the solvent nonetheless, as can be observed in the used baselines for the conduction of these analyses that are in annex, in appendix C. This interference is present in both DESs below 250 nm, where a sudden drop in absorbance is witnessed but further interference is noted between approximately 260 and 285 nm in the C8:C12 DES. The obtained disturbance below 250 nm can only be due to the presence of C8 since it is the only common denominator in both used baselines and meanwhile the interference between the specified wavelengths in the C8:C12 DES can only be due to the presence of C12 because the HBD is the only altered variable in the used baselines as well as in the solubility tests; this interference can be seen in the next figure.

The following chart allows us to compare the obtained UV-vis absorption bands by superposing the two spectrums.

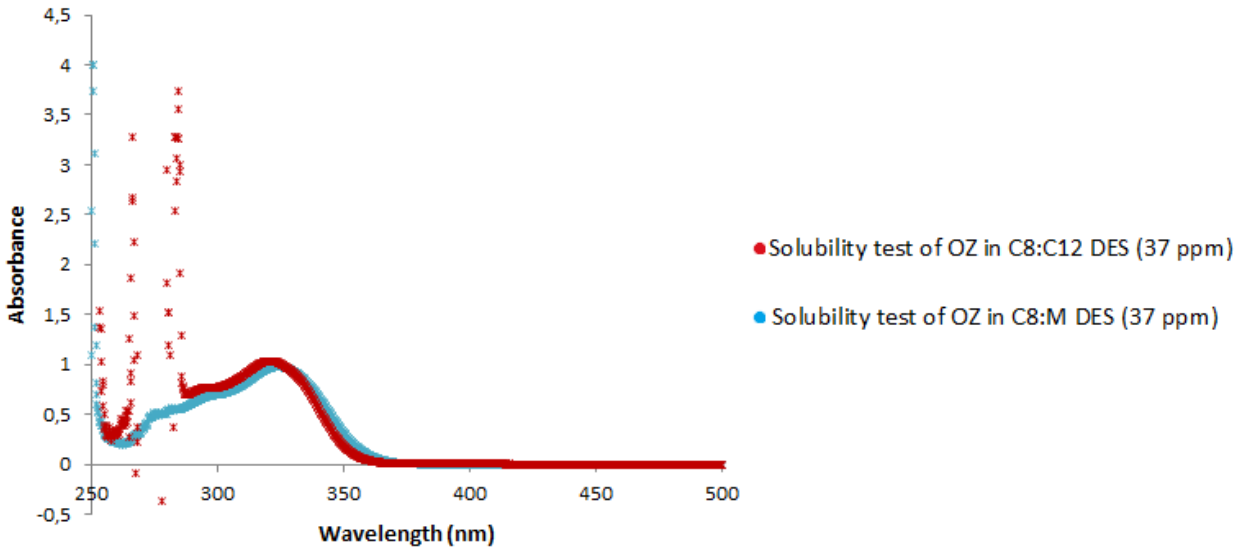


Figure 11 - Comparison of the solubility tests' UV-vis spectra.

As aforementioned, the C8:C12 DES matrix interference in the signal is apparent in the wavelength range of 260 to 285 nm but it must be underlined that this region is not crucial for determining the presence of OZ because it does not contain the absorption band's maximum. With this in mind, it can be observed that both spectrums are similar, with only a slight deviation of 10 and 5 nm in the C8:C12 DES and the C8:M DES, respectively, from the 330 nm reported maximum; indeed a wavelength shift of the maximum can be noticed in the figure due to the specific interactions of each solvent with OZ, allowing us to conclude that although similar, both solvents have different affinities for OZ.

In respect to the assays of the OZ extraction from rice straw and before introducing their preliminary analysis, it is worth mentioning that their filtration was crucial to obtain a DES-rich extract phase without any trace of biomass sample, allowing for their posterior handling; the difference in turbidity of the solution is presented in the next figure.

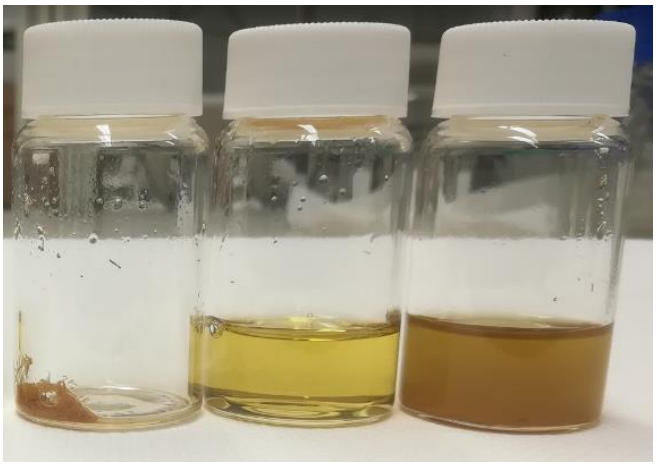


Figure 12 – Aspect of the DESs' extraction assays: left, biomass used; center, centrifuged and filtered; right, centrifuged only.

A significant difference can be seen between the solely centrifuged (on the right) and the centrifuged and filtered sample (in the middle), suggesting that the centrifuging step of the employed methodology can be skipped, leading to lower energy consumption and consequently, higher process efficiency.

In the next figure a comparison between the UV-vis spectra of the OZ solubility test and the respective OZ extraction assays from rice straw is presented.

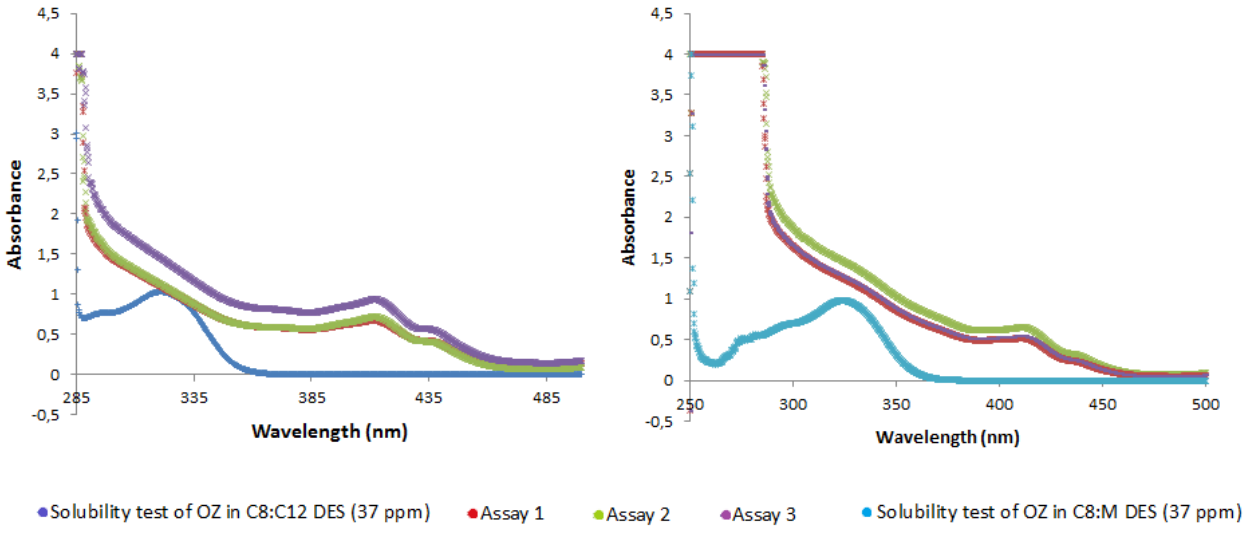


Figure 13 - Comparison of the UV-vis spectra of the OZ solubility test and OZ extraction from rice straw biomass using two hydrophobic DESs.

The obtained UV-vis spectra of the OZ extraction assays using both DESs show that the ferulic acid characteristic absorption band cannot be identified and therefore the use of an HPLC is mandatory. This is

probably due to the simultaneous extraction of other compounds present in the rice straw besides OZ. Nevertheless an inflexion point can be noticed in all the assays around the 330 nm mark, indicating the possible existence of OZ in the extracts.

Another important remark is that only a non-selective extraction of the OZ's compounds is achieved with this simple methodology; from an analytical perspective, the sole use of cycloartenyl ferulate as a standard does not hinder this evaluation as it was reported in the studied literature[111] that all of OZ's individual components have similar absorption curves with the highest absorbency at a wavelength of 330 nm.

Furthermore, the slight differences between the replicas can be justified by the sample variability, commonly observed when using lignocellulosic biomass. The triplicates' results are overall, all in good agreement with one another.

A comparison of two representatively chosen UV-vis spectra, each one corresponding to the extraction of OZ with each DES, can be found in the next figure.

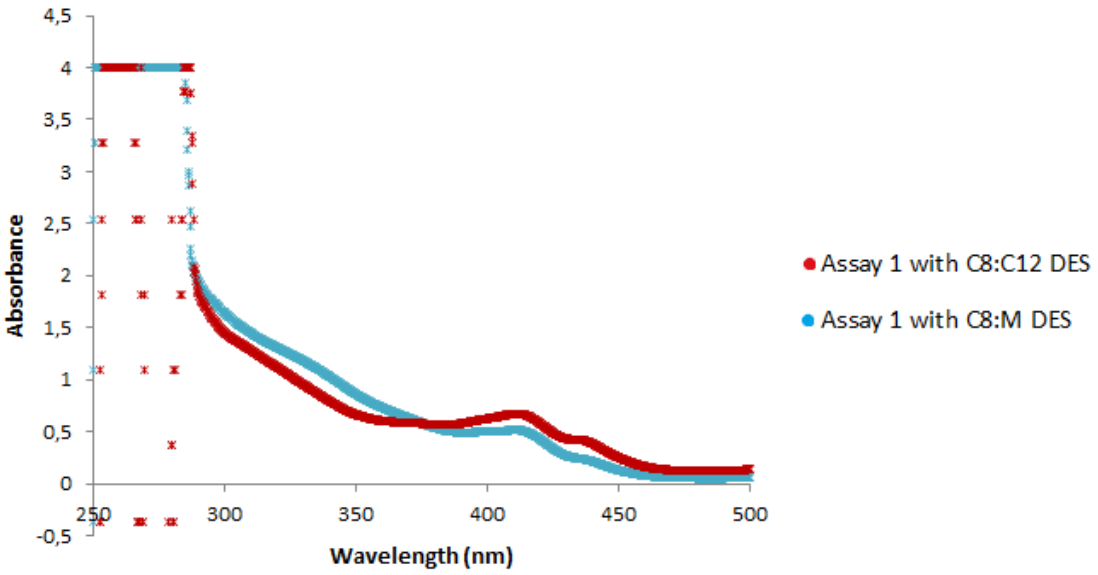


Figure 14 - Comparison of the extraction step's experiments' spectrums.

As was previously mentioned, apart from the interference of the C8:C12 DES around the 270 nm region, both assays lead to similar results. However, it can be observed that above approximately 380 nm, the C8:M DES extract has a lower absorbency than the C8:C12 DES, implicating a lower extraction efficiency, while in the region between 380 to 290 nm, the opposite is verified, therefore suggesting that the former DES has a higher extraction efficiency of OZ. This is paramount as each DES seems to present a higher extraction selectivity for certain compounds.

Moreover, the saturation observed below 290 nm attests for the extraction of other lipophilic material contained in the rice straw. In fact over 60 compounds fitting this description exist in this lignocellulosic biomass and the literature reports the use of a similar wavelength for detecting them[135], suggesting that their absorption bands have maximums around this region which could in turn explain the observed spectrum saturation. Although these compounds could bring forth further valorization of this process and of the general employment of rice straw as a source of valuable extractives, this study does not delve on their analysis and focuses solely on the detection of OZ.

It must also be stated that the extraction of one or more rice straw's constituents responsible for its color was achieved, as was verified by the yellowish color of both DESs after the extraction procedure; this is indeed validated by the presence of two observed peaks in the 415 to 430 nm region, associated with the visible region, that should correspond to these compounds.

Regarding the different affinity of both DESs for OZ observed in the solubility tests' in the form of a wavelength shift relative to the expected maximum, this was not observed in this previous figure.

The next section presents the HPLC analysis of the extraction assays, carried out to quantitatively infer on the OZ extraction yield of each used DES as the preliminary UV-vis spectrophotometry was inconclusive.

3.1.3 OZ Extraction Analysis

The HPLC analyses presented in this section are those of the OZ extraction assays carried out with the two chosen DESs and hexane for comparison purposes. The obtained chromatograms are shown in the next figures allowing the quantification of OZ in the extracts. The needed calibration curve, made using OZ in hexane, is presented in the next figure.

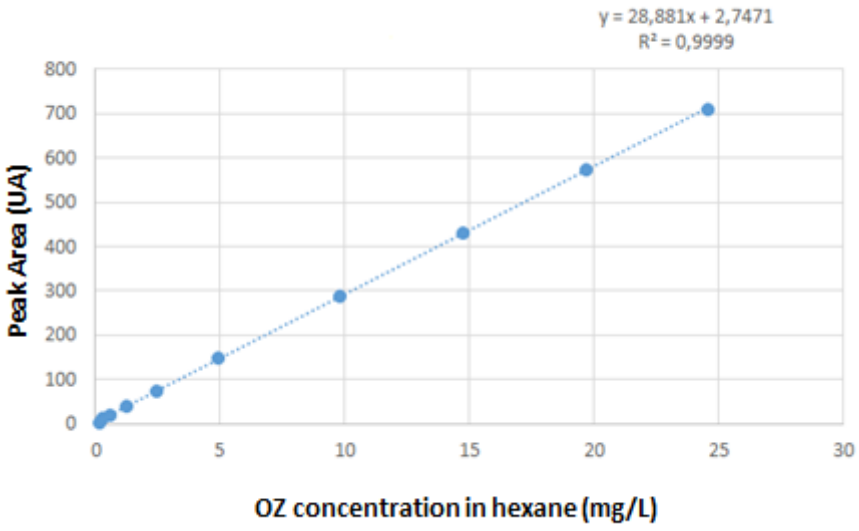


Figure 15 - Peak area in function of OZ concentration in hexane.

It can be seen that a linear relation between the OZ's peak area and its concentration in the solvent is obtained, thus making this quantification method appropriate for the determination of its content in the extraction assays. Moreover, the choice of hexane as the standard's solvent is due to the fact that the mobile phase in the HPLC analysis is composed of 94 v% hexane and the obtained retention time of OZ in the column was 4.65 min. The chromatograms corresponding to the calibration curve are presented in annex, in figure 34.

In the following figures, a representatively chosen chromatogram from each extraction assays' series, as they were performed in triplicates with the C8:M, C8:C12 and hexane, is presented. The chromatograms referring to the other extraction assays of OZ from rice straw with the chosen solvents are presented in annex, in appendix C.

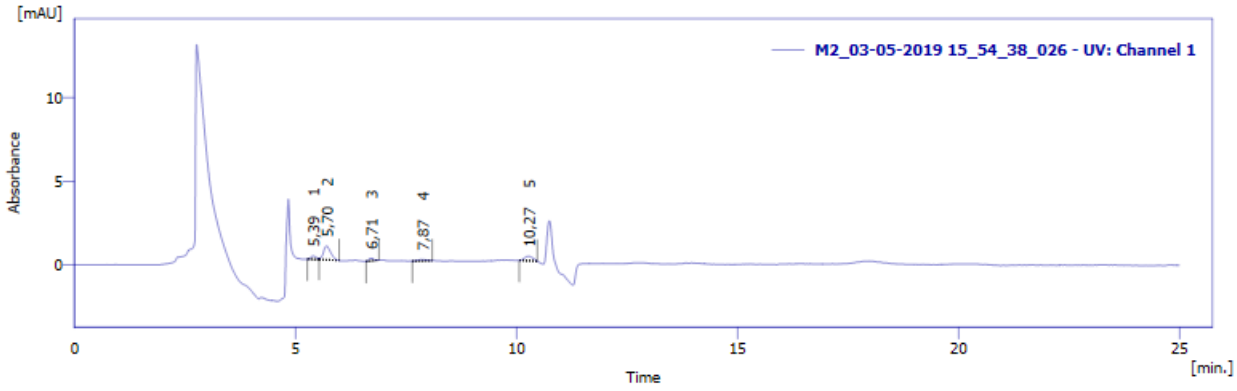


Figure 16 - HPLC chromatogram of an extraction assay with the C8:M DES.

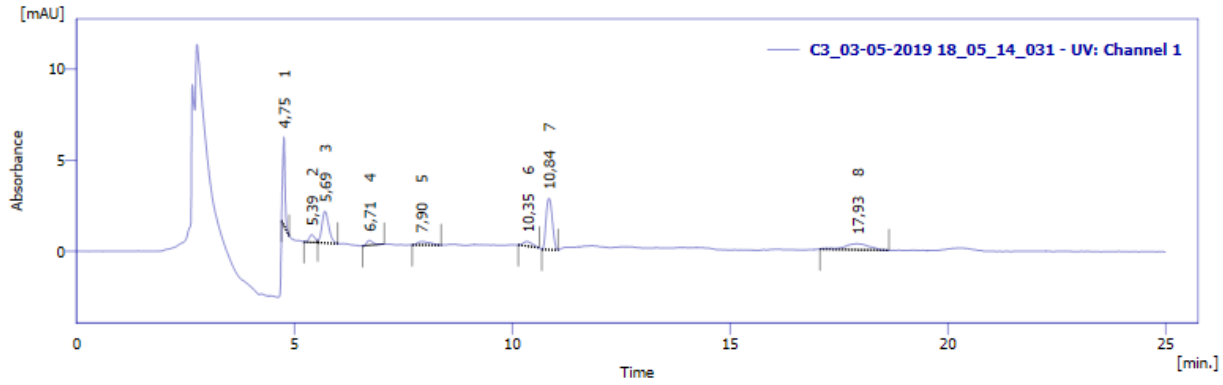


Figure 17 - HPLC chromatogram of an extraction assay with the C8:C12 DES.

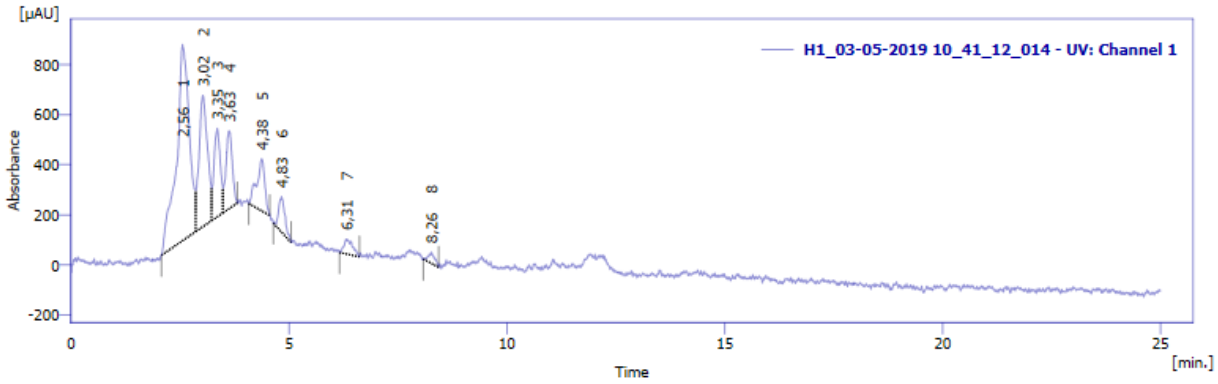


Figure 18 - HPLC chromatogram of an extraction assay with hexane.

In all of the above chromatograms, the absence of OZ's characteristic peak at the 4.65 min mark is noted thus concluding that no OZ was extracted with hexane and also suggesting that it could not be extracted with the tested DESs as well. However as the solvent's matrix can influence the retention time of OZ in the column, an addition of the OZ standard to the extraction assay of both DESs and the obtained chromatograms are hereby presented.

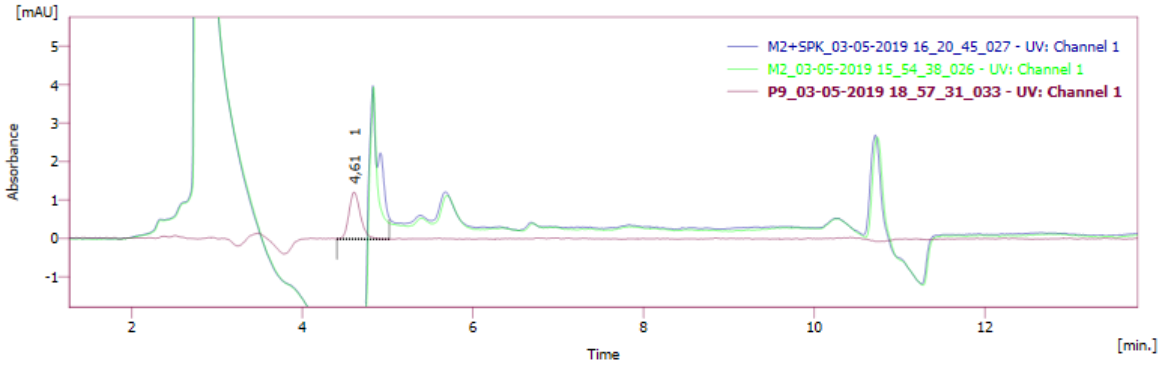


Figure 19 - Comparison of HPLC chromatograms obtained from an extraction assay with C8:M DES with (in blue) and without (in green) the addition of OZ standard.

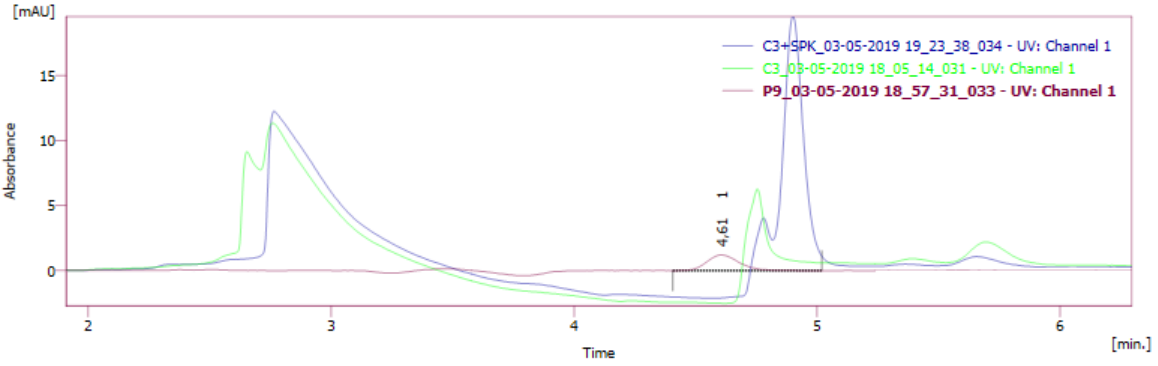


Figure 20 - Comparison of HPLC chromatograms obtained from an extraction assay with C8:C12 DES with (in blue) and without (in green) the addition of OZ standard.

Indeed, the presence of the OZ's peak at 4.92 and 4.90 min in C8:M and C8:C12 chromatograms, respectively, after the addition of the pure OZ, leads to the conclusion that despite the matrix interference, as noted by the different observed retention times of OZ in the column in each assay, it was not the reason justifying the previous absence of its peak.

Thus, in conclusion, the extraction of OZ from rice straw was not effective as no OZ peak could be identified in the HPLC analyses. This could either be due to the very small concentration of this compound in the rice straw or to the fact that the protocol's experimental conditions were not optimized. Nonetheless the failure on extracting OZ with hexane, points to the former.

However, a plethora of other unidentified compounds were extracted not only with hexane, but also with the employed DESs, warranting further research on the identity of these substances for developing a sustainable valorization process of rice straw.

The next section evaluates the DESs' recyclability, had the extraction of OZ or a valuable compound been successful.

3.1.4 Analysis of the DESs' Recyclability

In this section, the residual biomass obtained was dried in an oven for at least 3 days with mild heating of 45°C to determine if the residue could indeed be dried in order to facilitate its posterior treatment in the next fractionation step. This would ease the residual biomass sample handling as it would provide an efficient method to remove the solvent. Additionally, this would mean that the DES could be evaporated in the DES-rich liquid extract phase as well, allowing the simple and efficient recovery of a purified valuable compound fraction. The following table summarizes the results of this experiment.

Table 9 - DES evaporation from the residual biomass solid phase obtained from the extraction experiments.

DES	DES evaporation (V-yes, X-no)
C8:C12	X
C8:M	V

It can be seen that only the biomass sample treated with C8:M DES completely dries under the experiment's conditions, demonstrating the feasibility of applying a simple drying step to the processual residue obtained with this solvent. Nonetheless it was not evaluated if the DES could indeed be evaporated or solely part of its constituents. Further investigation must be conducted, although the similar boiling points of C8 and M suggest that the whole DES is evaporated. If this is true then the recycling of this DES is possible, allowing for its recovery and usage in another extraction cycle while obtaining a dry residue that can be directly submitted to the posttreatment stage. Moreover, a purified valuable compound fraction can also be obtained from the DES-rich extract phase obtained with this solvent.

It must be noted that this drying step can be accelerated by increasing the temperature but a more extensive economic analysis must also be conducted as there would be a trade-off between the time it takes to evaporate the solvent and the oven's energy consumption during this period. Furthermore this result presents an exception to the general consensus found in the literature about DESs, stating that they are not volatile.

As for the C8:C12 DES, its recyclability must involve an alternative method as no noticeable solvent evaporation was observed in the biomass sample, implicating that the DES is either not volatile or that harsher drying conditions need to be applied. Its application in this process would require, for instance, washing the residual biomass sample with a non-polar solvent to remove it and further drying of the residue to prepare the biomass for the next valorization step. Although the drying step could be more efficient, this proposed alternative would lead to higher costs and further waste generation due to the employment of another chemical agent. Regarding the C8:C12 DES-rich extract phase and the recovery of a purified valuable compound fraction from it, this would no longer be possible without the addition of another substance that could either act as an anti-solvent, thus precipitating the valuable compounds, or as a better solvent therefore carrying out a liquid-liquid extraction.

The next section proceeds to evaluate the potential of developing a posttreatment for the residual biomass sample obtained from the extraction experiments. It must be underlined that this assessment is conducted with fresh rice straw samples that were not submitted to the extraction protocol.

3.2 Hydrophilic DES Treatment: Fractionation of Rice Straw Biomass

This next part of the results' section consists on the analysis of the results from the development of a rice straw valorization process with the objective of producing two LVHV products, namely a high fermentable sugar content liquid and solid residue. This part involves the choice of DES concentration in the employed DES aqueous solutions, followed by a screening of their rice straw dissolving capabilities and finally their fractionation results.

3.2.1 Choice of DES Concentration in DES Aqueous Solutions

As was previously discussed, the DES concentration in the DES aqueous solution is paramount in order to achieve a greater solubilization of rice straw and in turn a better depolymerization yield of the lignocellulosic biomass sample. Rice straw dissolution tests were performed to conclude on the effect of the water fraction in the DES aqueous solutions on the solubility of rice straw. The results of these tests in the CC:FRU and the CC:AA aqueous DES solutions are presented in the next tables. The experiments were conducted at 60 and 90^{o}C, according to the previously described protocol, and the DES formed in aqueous media is indicated by the *, being only used in the experiments at 60^{o}C.

Table 10 - Rice straw solubility at 60°C in the two chosen DESs.

	Rice straw solubility in the DES (mg/g DES)	
Water in DES aqueous solution (wt%)	CC:FRU	CC:AA
10	0.62	0.76
20	0.94	0.72
30	0.47	0.75
40	0.42	0.76
50	0.43	0.77
20*	0.93	0.73

*- DES formed in aqueous media

Table 11 - Rice straw solubility at 90°C in the two chosen DESs.

	Rice straw solubility in the respective DES (mg/g DES)	
Water in DES aqueous solution (wt%)	CC:FRU	CC:AA
10	[5 – 6]	[4 – 5]
20	[5 – 6]	[3 – 4]
30	5	[3 – 4]
40	[4 – 5]	[3 – 4]
50	[4 – 5]	[3 – 4]

During this experiment the CC:FRU DES gained a brown color. This can be seen in the next figure.

Figure 21 - CC:FRU DES displaying a change in color.

The obtained solubility at 90°C of approximately 6 mg/ g of DES is in agreement with the literature's result[91] as the observed tendency from these experiments is the increase of the rice straw solubility with the increase of temperature. In fact, the solubility results obtained at the two temperatures are very different from each other indicating the relevant role this variable plays in the dissolution of rice straw biomass in the chosen DES.

It can be observed that solubility of biomass is particularly favored at higher temperatures (90°C), with an increase of order of magnitude. It can also be observed that at 90°C highest solubility is afforded by CC:FRU, while slightly lower solubility is achieved with CC:AA. The inverse order was found at 60°C with the sole exception of the CC:FRU DES aqueous solution with a 20 wt% water content managing a better performance.

Looking at the DESs' chemical structures, these two DESs interact differently with the lignocellulosic biomass's constituents. While the CC:FRU DES may form more hydrogen bonds with the polymeric saccharides present in the sample due to its hydroxyl groups, the CC:AA DES's acidity should allow for a better digestion of the rice straw, thus freeing its components and leading to a higher solubility as well. Nonetheless, the results show that the CC:FRU mixture is more efficient at solubilizing rice straw than the CC:AA DES, suggesting that its mechanistic interaction with the biomass is more favorable. And yet, the FRU's hydroxyl groups that give the DES its advantages, also turn into a problematic matter at the temperatures of these experiments; the polymerization of the FRU, most likely into fructan, that can be observed in the presented picture where the solvent displays a caramel-like color, led to the complete solidification of the DES in under a week. This is enabled by the hydroxyl groups' reactivity. As such, although the CC:AA DES has a lower rice straw dissolving potential, its thermal stability and easier handling make it a more suitable candidate for this process.

In what concerns the effect of water on the rice straw biomass dissolution, it can be observed that a small water fraction leads, in general, to a higher biomass dissolution, for both DESs and both studied temperatures, justifying the choice of a 10 wt% water content in the DES aqueous solutions in the next stages of this work. Furthermore, the results for CC:AA indicate that the dissolution process carried out in this DES is less affected by the water content, unlike the CC:FRU DES, where the increase in the water fraction manages a twofold increase of rice straw solubility at 60°C. This is due to the fact that these DESs are very viscous and the addition of water facilitates the mass transport.

3.2.2 Screening of Hydrophilic DESs for Biomass Treatment and Fractioning

Now that the optimal water fraction in the DES aqueous solution was determined, a screening of 8 DESs listed in the table 8 in the DES preparation section, for the rice straw biomass solubilization at 25 and 50°C, is presented and discussed in this section.

The following pictures depict the screening experiment that was carried out in an oil bath with magnetic stirring at 25°C and in a thermomixer at 50°C.

Figure 22 – Experimental set up to screen DES's ability to solubilize rice straw biomass.

The next table presents the results of this screening at 25°C.

Table 12 - Observations made at defined time periods during the screening for rice straw dissolution at 25°C. For sake of simplicity, DESs are here referred to using the number assigned in table 8 in the DES preparation section.

	Observation 1 (6 days in)	Observation 2 (10 days in)	Observation 3 (14 days in)	Observation 4 (20 days in)	Observation 5 (1 month and 1 week in)
Granules	Presence in all DESs is noticeable with less abundance in 1	Same as before	Presence in all DESs with slightly less abundance in 1	Noticeable presence in all DESs but 1 presents almost none	Same as before
Granules on the surface of the liquid, suspended or decanted	Suspended in all DESs except for 7 and 8 where they are on the surface	Same as before	Same as before	Same as before	Same as before
Granule size	Original size but thinner in 1	Same as before	Thinner particles in all DES except for 6, 7 and 8	Extremely thin in 1, very thin in 2, 3 and 4 , thin in 5 and 6, original in 7 and 8	Extremely thin in 1, 2, 4 and 6, very thin in 3 and 5, original in 7 and 8
Color of granules	Original color in all except 1 and 2 where they are colorless and	Colorless in 1, yellowish in 2, pale brown in 3, 4 and 6, original color	Colorless in 1, 3 and 4, yellowish in 2, pale brown in 5 and 6,	Colorless in 1, 3, 4 and 6, yellowish in 2, pale brown in 5, original	Colorless in 1, 3, 4, 5 and 6, yellowish in 2, original color in 7 and 8

	slightly yellowish, respectively	in 5, 7 and 8	original color in 7 and 8	color in 7 and 8	
Presence of precipitate	None	None	None	None	In 1 and 4 a small cloud appeared
Precipitate's color	-	-	-	-	Pale white in 1 and 4

From the observations made throughout a one month period of this screening at 25°C, it can be concluded that the DESs 7 and 8 presented the worst results. Corresponding to the CC:TA and the CC:CA DESs, their high viscosity did not allow for a proper solvation of the rice straw sample, conducing to an improper dissolution. In fact, during the entire period of time, the rice straw granules appeared on the surface of these DES although constant stirring at 400 rpm was applied. As for the other DESs, 1, 3 and 4, corresponding to CC:AA, CC:PEG 200 and CC:LA, presented the most promising results with colorless particles by the second week of the experiment and very thin granules by the 20 day mark, with DES 1 and 4 distinguishing themselves from the rest due to the appearance of a small precipitate in solution at the end of the experiment; this could attest for a higher acidic digestion of the biomass conducing to a higher release of its components and thus saturating the DES. Furthermore, the CC:U DES assay, noted as DES 2, showed interesting results as its granules turned from brown, the original rice straw sample color, into yellow, showcasing an interaction of the solvent with the constituents that give the biomass its coloration.

Although no tested DES managed to dissolve the added biomass, resulting in rice straw solubilities that were smaller than 0.5 mg/g of DES, favorable results for the further selection of the fractionation DESs were still obtained, as each solvent presented different degrees of rice straw solubilization, varying in efficacy.

The next table presents the results of this screening at 50°C.

Table 13 - Observations made at defined time periods during the screening for rice straw dissolution at 50°C. For sake of simplicity, DES are here referred to using the number assigned in table 8 in the DES preparation section.

	Observation 1 (24 hrs after)	Observation 2 (5 days in)	Observation 3 (16 days in)	Observation 4 (23 days in)	Observation 5 (1 month in)	Observation 6 (almost 2 months in)
Granules	Presence in all of the studied DESs with slightly less abundancy in 5, 6, 7 and 8	Presence is notable in all DES except in 4 and 6 where they are harder to see	Presence is notable in all DES except in 4 and 6 where they are much harder to	Same as before	Same as before but 6 no longer presents any visible discreet particles	Same as before

			see			
Granules on the surface of the liquid, suspended or decanted	Suspended in all DESs except for 5,7 and 8 where they are on the surface	Suspended with a few decanted except in 5,7 and 8 where they remain at the surface	Decanted in 1, 2 and 3, suspended in 4 and 6, on the surface and some suspended in 5,7 and 8	Same as before	Decanted in 1, 2, 3 and 4 while on the surface and suspended in 5, 7 and 8	Decanted in all DESs except 6
Granule size	Original	Similar to original except in 4 and 6 where they are smaller	Similar to original except in 4 and 6 where they are extremely thin	Same as before but 1, 2 and 3 present slimmer granules	Original in 5, 7 and 8, thin particles in 1, 2 and 3 while extremely thin in 4	Original in 5, 7 and 8, very thin particles in 1, 2 and 3 while extremely thin in 4
Color of granules	Original	Original	Colorless in 4 and 6, pale yellow in 2 and a pale brown in the rest	Colorless in 1, 3, 4 and 6, pale yellow in 2 and pale brown in 5, 7 and 8	Same as before	Same as before
Presence of precipitate	None	None	None	None	None	In 2 and 4 a small cloud appeared
Precipitate's color	-	-	-	-	-	Pale yellowish in 2 and pale white in 4

In respect to the screening conducted at 50^{o}C, it must be remarked that the use of 1.5 mL Eppendorf containers was detrimental to the mass transfer between the solvent and the sample due to bad mixing, although the higher temperature promoted a decrease in viscosity. This is particularly apparent in the DES 5, corresponding to CC:MA, wherein the previous experiment the granules managed to be suspended in the solution as soon as the first observation was made while taking almost two months in these conditions to achieve the same; not to mention DES 7 and 8 where once again the solvation effect of the solvent was limited due to the lower mass transfer area. On the other hand, DES 1, 2 and 4 fared even better than in the previous experiment, with the rice straw granules turning yellow in the CC:U DES and colorless in the others while also being present in fewer numbers and with a thinner appearance. Regarding the formed precipitates in solvents 2 and 4, the same reason as before can be invoked for its appearance in the latter whereas the precipitation of the solubilized components in the CC:U mixture means that this DES reached the thermodynamic equilibrium faster, therefore presenting a higher rice straw dissolution rate than the other DESs, apart from the CC:LA; this is corroborated by the fact that once again in this experiment the major part of the tested DESs did not dissolve

the added biomass. The resulting rice straw solubilities were inferior to 0.5 mg/g of DES with one exception; DES 6.

In fact, in this screening at 50^{o}C, the CC:OA DES managed to dissolve the added lignocellulosic biomass sample, outperforming all of the other DESs. No further addition of rice straw sample was carried out for the sake of comparing the solvents using the same concentration.

In conclusion, the four more interesting DESs (1, 2, 4 and 6) from these tests were chosen to carry out the fractionation step of the biomass with the goal of obtaining the two LVHV products.

3.2.3 Fractionation of Rice Straw

Finally in this stage of the work, the four selected fractionation DESs were applied in the treatment of the rice straw biomass for achieving a residue rich in fermentable sugars that can then be converted into valuable products, as biofuel for instance, by a fermentation or enzymatic hydrolysis. Therefore, a depolymerization of the polysaccharides contained in the sample is sought for. The applied procedure is described in the hydrophilic DES treatment section. However, it must be stated that a slight modification in the original DES selection, composed of CC:AA, CC:U, CC:LA and CC:OA, was needed; the CC:OA DES was replaced by the CC:MA. This is due to the degradation of OA into hydrogen and carbon dioxide at the process temperature[136], making the employment of this DES unfeasible in this step. Nonetheless, its use is reported in the article studied in the literature review section for this treatment under the employed conditions. The following image depicts the observed degradation of the CC:OA DES thus warranting its exclusion from this section.

Figure 23 - CC:OA DES degradation in the fractionation step.

It can be seen that all the assays were contaminated and that the CC:OA DES aqueous solutions leaked out of the flasks possibly due to the pressure build-up caused by the gas formation resulting from the OA's degradation.

Thus the aforementioned selected DESs were used in order to solubilize the lignocellulosic biomass sample and two phases were obtained: a solid phase consisting on the Büchner filtration's cake, that was then dried and weighed to infer on the effective solubilizing ability of this methodology, and a liquid consisting on the filtrate from the same filtration, that was analyzed by HPLC to infer on its monomeric sugar content. These LVHV products are considered residues in the global valorization process that is developed in this thesis and are referenced as such, although they could be considered as products of this section's subprocess.

3.2.3.1 Recovered Solid Residue

The following figures present the residue recovery fraction calculated as the weight ratio of the recovered undissolved biomass by the added original sample and the achieved rice straw concentration in the DES aqueous solution.

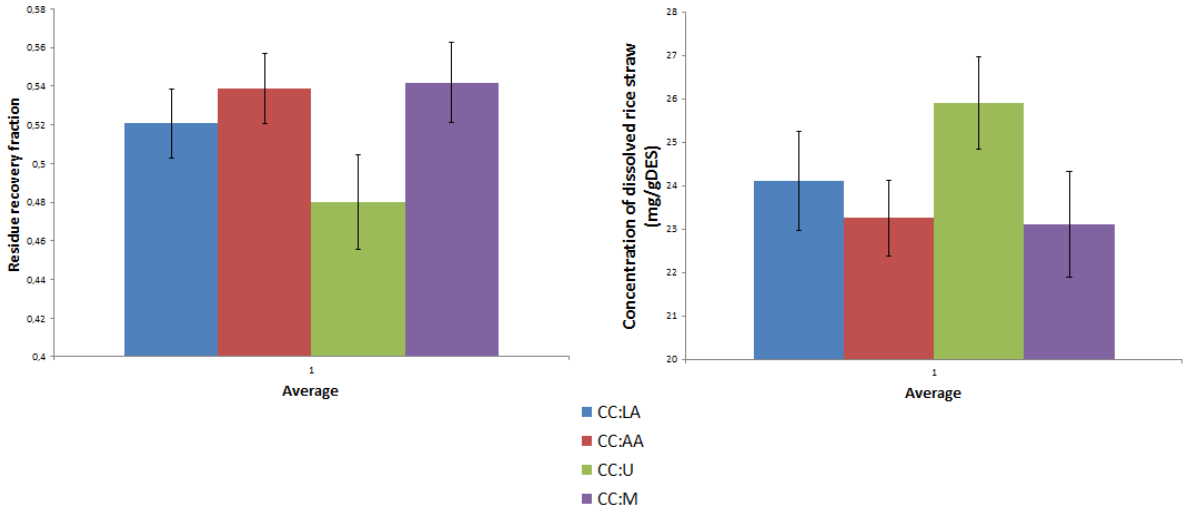


Figure 24 - Residue recovery fraction and concentration of dissolved rice straw with each tested DES for incubation at 120°C, 18 hrs (+/- 1 S.D.).

The employed protocol involved the use of a high process temperature of 120°C for 18 hrs to not only guarantee that the solvent saturation is reached but also to allow the thermal degradation of the biomass sample. It was based on a methodology employed in the literature[104] where it could be observed that for the proposed one step treatment, higher processing times led to lower residue recovery fractions and therefore higher rice straw concentrations in the DESs.

This is exactly what can be observed in our experiments. An even higher solubility of rice straw in the DESs is achieved in this presented method thus implicating a further depolymerization of its constituents since their polymeric form hinders their dissolution in these hydrophilic solutions. Furthermore, the drastic solubility increase achieved in this process outperforms the other comparable literature result of the dissolution of rice

straw in the CC:FRU DES at 120^{o}C, where a solubility of 6.5 mg/g of DES was afforded in a simple dissolution test[91].

In respect to the presented charts, it can be seen that the CC:U DES showcased the best performance in terms of both residue recovery fraction, with an average recovery of 48% of the originally added rice straw, and biomass concentration in the solution, amounting to an average of 25.9 mg/g of DES. The obtained solid residue with this DES's employment was less than with other solvents thus inferring a greater dissolution of rice straw in it. It should be added that in contrast with the screening's results, the rice straw's yellowish color was not noted. As for the other DESs, no statically significant difference in these factors was observed between them. An explanation for these observed results may be the fact that U can be both a HBA and a HBD; it could be hypothesized that this characteristic ability permits a greater interaction with the lignocellulosic biomass's constituents thus leading to a more significant depolymerization and subsequent solubilization.

It must be remarked that as the literature[104] did not explicitly state the temperature at which the water was added to the assay, nor its volume in the posttreatment water addition stage, an arbitrary volume and temperature of 20 mL and 68^{o}C were chosen. The variation of these parameters was not studied but they most probably are of utmost importance as the water addition to these kinds of matrixes can lead to very different outcomes, as seen in the literature review section. Further analysis on the composition of these solid residues is also needed to completely assess the effectiveness of the depolymerization since its contents in cellulose, hemicellulose and lignin would be greatly informative, as each solvent has a different affinity for the used biomass's constituents and the mechanisms involved in their dissolution are all different. These analyses could have been obtained through the application of the Van Soest method or the NREL's procedure but alas they were not carried out due to the lack of the needed apparatus.

The following figure shows the effect of the water fraction on the studied parameters with the CC:AA DES.

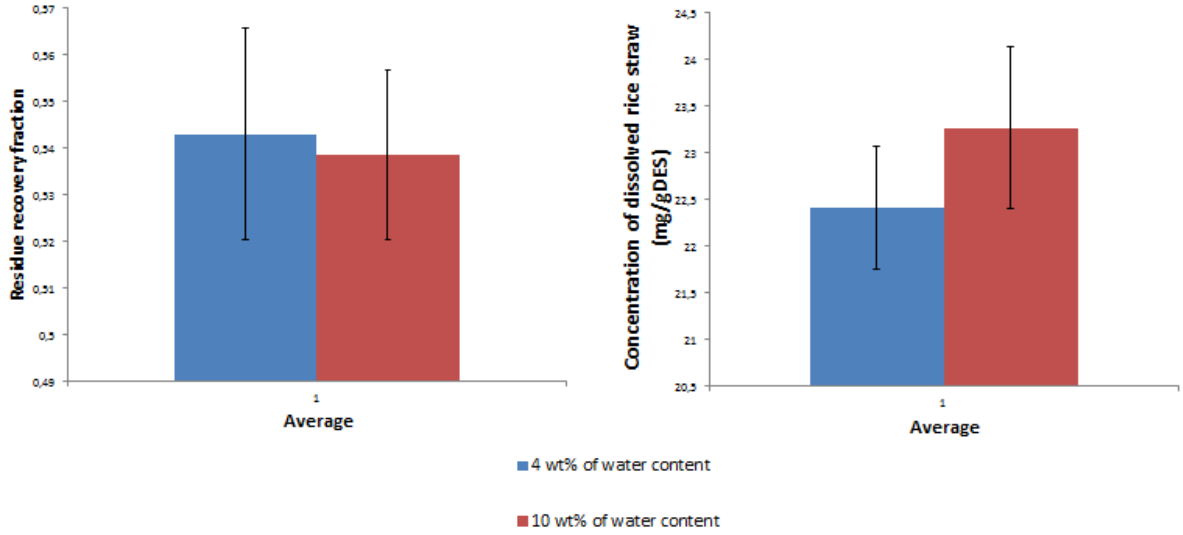


Figure 25 - Water effect on the residue recovery and on the concentration of dissolved rice straw with the CC:AA DES (+/- 1 S.D.).

This experiment was conducted to bring more insight on the observations made during the experiments on the choice of DES concentration in the aqueous solution, as in this mentioned section the water content of the solution did not affect the performance of the CC:AA DES.

It can be seen that no statistically significant difference is observed in the residue recovery fraction and in the concentration of rice straw in this DES thus validating the previous observations. This could be due to the fact that the minimum water fraction threshold to warrant an efficient solubilization of rice straw is attained and while not being evident in the presented charts, it is particularly apparent in the next figure that presents the effect of the water fraction on the studied parameters with the CC:U DES.

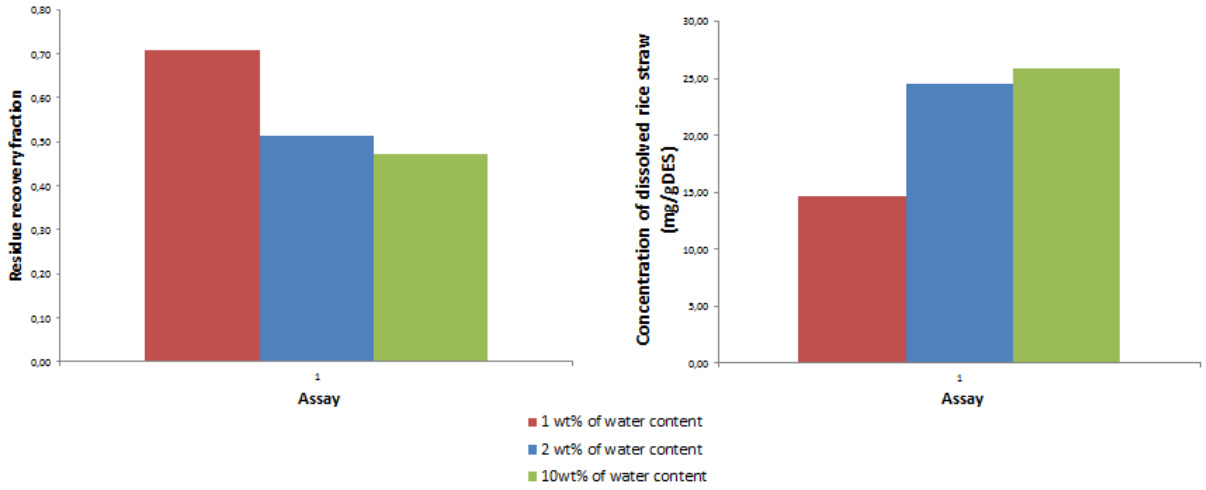


Figure 26 - Water effect on the residue recovery and on the concentration of dissolved rice straw with the CC:U DES.

As for the CC:U DES, it can be seen that the water content in the DES aqueous solution does indeed influence the results: a significantly higher degree of biomass solubilization is achieved in the 2 and 10 wt% water content assays. This is due to the decrease in viscosity in the DES aqueous solution that was visually noticeable during the conduction of these experiments. As already discussed, a higher viscosity leads to a hindered mass transfer and other associated rheological dissolution impediments.

Furthermore, the reduction of the residue recovery fraction that can be observed from the 1 to 2 wt% water content assay is considerably more significant than the one observed from the 2 to 10% water content assay, meaning that although the use of a 10 wt% water fraction DES aqueous solution can be justified for achieving a lower residue recovery fraction and a higher rice straw solubility, the use of a DES aqueous solution with only a 2 wt% water content practically achieves the same result, all the while giving an economical and eco-friendly advantage to this developed process. Thus, the aforementioned threshold appears to be at 2 wt% for the CC:U DES. It must also be mentioned that in this type of lignocellulosic biomass's treatment with DESs, the increase of the water fraction in the solution can lead to the dissociation of the HBD and HBA resulting in the decay of the process efficiency and so a maximum is expected[137]. Nonetheless, the fact that some authors debate the synergistic activity of both the dissociated DES's components in the water[95], as previously debated, leaves room for much more discussion on this topic as each DES seems to present a different case.

The next section presents the results from the HPLC analysis of the recovered filtrate to assess on its monomeric sugar content.

3.2.3.2 Recovered Liquid Residue

To the best of the author's knowledge, this is the first report on the use of DESs for rice straw treatment where a liquid residual phase was considered for valorization purposes and as far as could be determined, no comparable results in the literature exist since no investigation on this matter was ever reported. Nevertheless, reports on the carbohydrate content of pre-fermented solutions were useful to conclude on the effectiveness of this process.

The following figures present the concentration of the filtrate in glucose and xylose obtained with the different DES aqueous solutions.

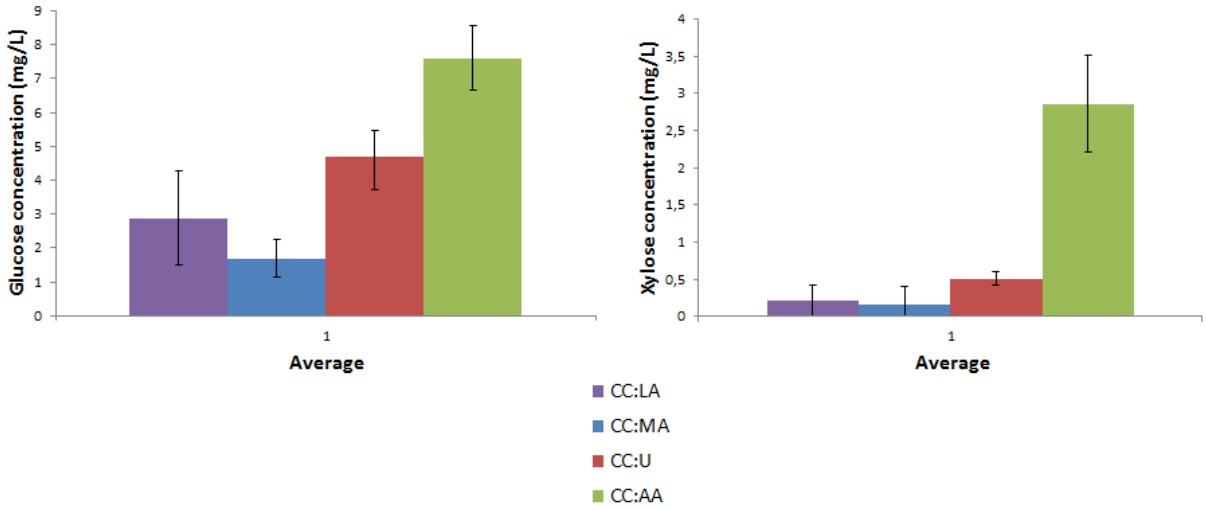


Figure 27 - Glucose and xylose yields in the liquid phase residues obtained from the fractionation step (+/- 1 S.D.).

In what concerns to the glucose yields, it can be seen that there is a statistically significant difference between the CC:AA DES and all the other DESs, with this mixture managing the best result of 7.6 mg of glucose/L of filtrate. As for the other DESs, the CC:U appears to be better at depolymerizing the rice straw than CC:MA and regarding the CC:LA DES's performance, the uncertainty makes it comparable to that of the CC:MA and CC:U DESs.

It is interesting to note that the aforementioned mechanism of the CC:AA DES for solubilizing the rice straw, involving its acidic character, does not appear to justify the observed results as the CC:LA DES that has a more acidic nature, due to the lower pK_A of the LA in comparison with the AA, does not achieve the same degree of depolymerization of the rice straw that the CC:AA DES did. It could be hypothesized that the additional hydroxyl group of the lactic acid could actually stabilize the lignocellulosic biomass's constituents by creating hydrogen bonds with them.

Before discussing the xylose yields, it must be stated that this rice straw's constituent originates from its hemicellulose as this fraction is composed of a xylan backbone[96][97][98], giving xylose when it is

depolymerized. Considering the obtained xylose concentrations, it can be seen that once again, the CC:AA DES manages the best performance with a result of 2.9 mg of xylose/L of filtrate followed by the CC:U DES that afforded a much more modest 0.51 mg of xylose/L of filtrate. The xylose concentrations resulting from the two other DESs' use is even lower, only amounting to approximately 0.18 mg of xylose/L of filtrate.

Consequently, although the CC:AA DES is not the best at solubilizing the rice straw as was previously seen, it does manage to selectively depolymerize more of the biomass's components hence creating a higher monomeric sugar content liquid residue that can then be directly fermented as the aqueous media is friendly to organisms who carry out these functions or dried to obtain a sugar-rich solid residue. Subsequently, the application of an enzymatic hydrolysis step to further refine these mentioned residues, both the liquid and solid, is feasible since their remaining saccharides are under a polymeric form thus hindering the organisms' fermentation activity; it must be noted that the liquid phase residue obtained in this step is also friendly to enzymatic activity since it is an aqueous media.

Furthermore when comparing these results with the literature's reports on the carbohydrate content of rice straw's pre-fermented hydrolysates obtained via conventional methods, where 41 g of glucose/L of hydrolysate and 21 g of xylose/L of hydrolysate were secured[138], it can be seen that both the glucose and xylose yields of this process are substantially lower. However, to truly compare these results, the obtained liquid fraction's enzymatic hydrolysis must be conducted because a non-negligible amount of polysaccharides may still exist in this residue and the employed analytical method only detects their monomeric form. This underlines the need of applying another treatment step to the obtained residue since this liquid phase is more comparable to an extract rather than to a hydrolysate. It should also be reiterated that the previously obtained solid phase is a promising source of fermentable sugars as well but its enzymatic hydrolysis is warranted to allow a comparison with these literature results.

The next figure presents the water's influence on this section's studied parameters in the CC:AA DES assays.

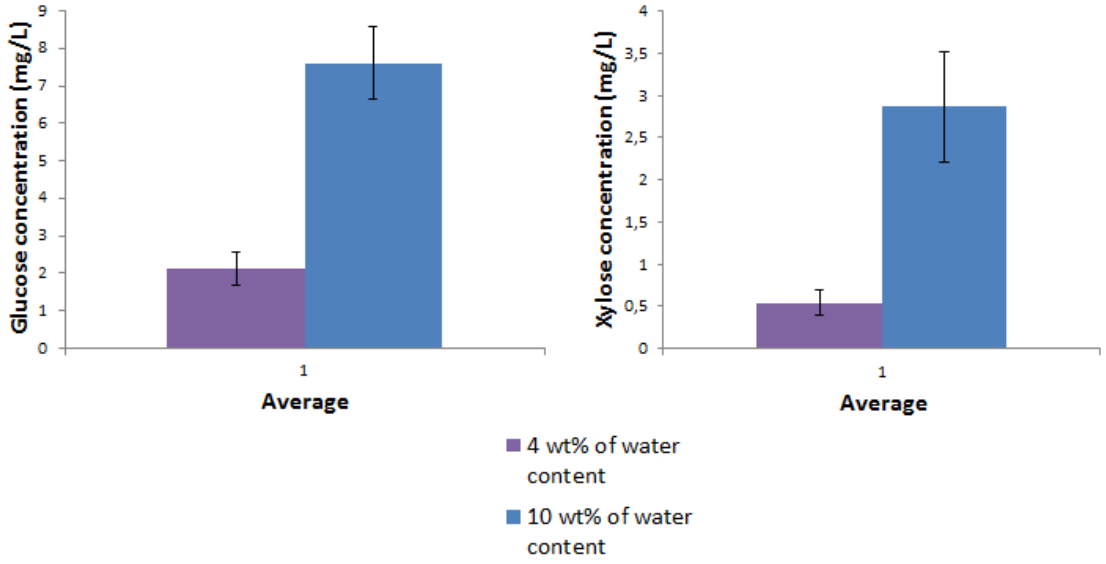


Figure 28 - Water effect on the glucose and xylose yields in the liquid phase residues obtained from the fractionation step with CC:AA DES (+/- 1 S.D.).

Unlike the non-existent water effect on the residue recovery fraction and the solubility of rice straw in the CC:AA DES, here the increasing water content leads to significantly higher glucose and xylose production, almost fourfold in the glucose yield and approximately sixfold in the xylose yield. This is due to the fact that water partakes in the hydrolysis of hemicellulose and cellulose, from where these sugars are obtained, as one of this depolymerization main reaction's reagents.

The next figure presents the effect of the water fraction on the glucose yield in the CC:U DES assays.

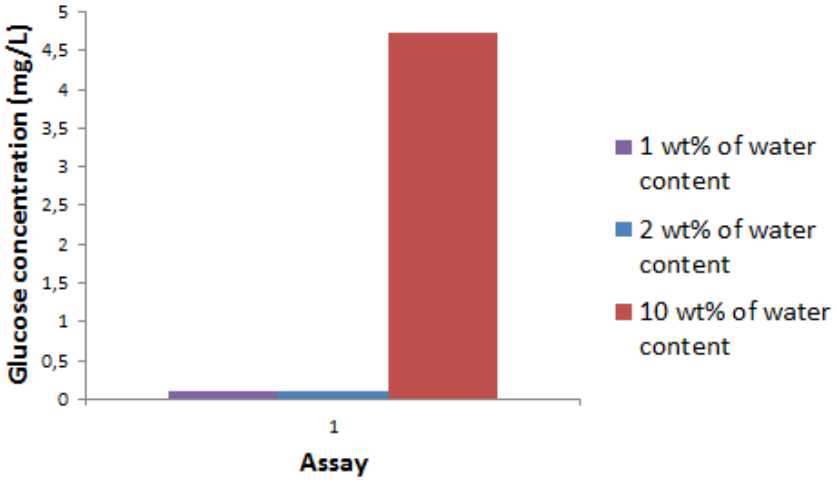


Figure 29 - Water effect on the glucose yield in the liquid phase residues obtained from the fractionation step with CC:U DES (+/- 1 S.D.).

In this case, besides being involved in the depolymerization reaction of the rice straw's components, water also lowers the viscosity of the solvent as previously discussed, leading to even better results. A drastic thirtyfold improvement in the glucose production is observed when water is present at a 10 wt% compared with a 1 wt%. The study of the water's effect on the xylose yield in this DES did not lead to appreciable results and no conclusions were taken.

In conclusion, it can be said that the presence of water is crucial for producing high monomeric sugar content liquid residues in this process and a water fraction of 10 wt% in the DES aqueous solution leads to a better performance over low water content solutions. In addition, a further study of the water effect, namely the use of high contents, could give a better insight on the optimal water concentration for achieving higher sugar content in these liquid residues.

The chromatograms where the study of these results was carried out are in annex, in appendix D, as well as the calibration curve that was used to determine the glucose and xylose concentrations.

3.2.4 Optimization of the Fractionation Step

The goal of this final section is to evaluate if promising results can be attained by using a smaller duration for the incubation of rice straw during this fractionation protocol. The two more promising DESs from the previous step were selected, being the CC:U and CC:AA, and the following figures showcase the results of this optimization.

3.2.4.1 Recovered Solid Residue

A comparison between the results of a 4, 8 and 18 hrs biomass incubation with the two tested DESs is hereby presented. It must be noted that the same parameters as before were monitored.

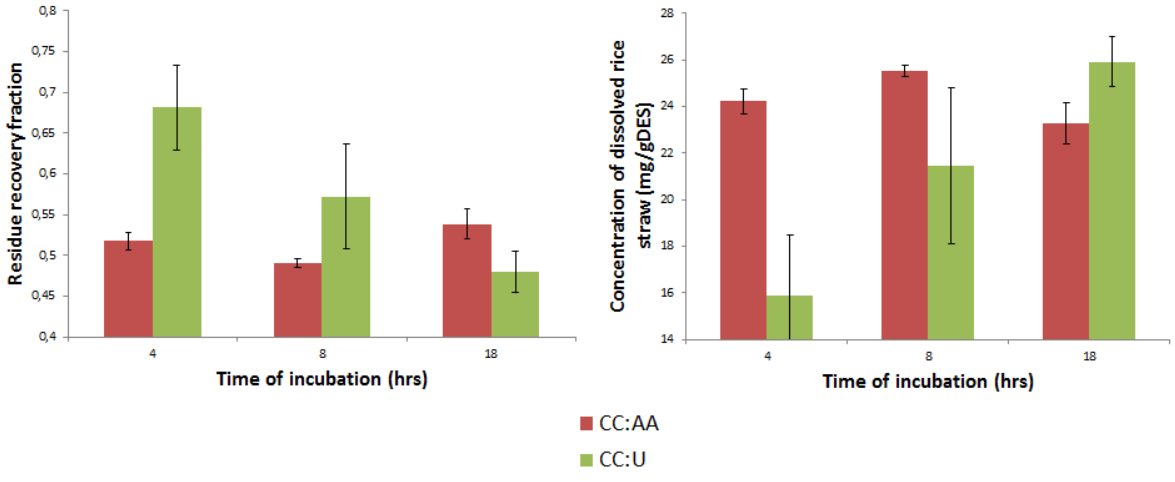


Figure 30 - Residue recovery fraction and concentration of dissolved rice straw with the tested DESs for incubation at 120°C during 4, 8 and 18 hrs (+/- 1 S.D.).

It can be seen that while the residue recovery fraction and the concentration of dissolved rice straw with the CC:U DES show significant improvements with the increase of the duration of this step, leading to the best results for the 18 hrs incubation, these studied parameters do not showcase the same tendency for the CC:AA DES. In fact, it would seem that the best results with this solvent are attained at the 8 hrs mark since a reduction of the residue recovery fraction and a subsequent augmentation of the solubilized biomass are noted in the passage from the 4 to 8 hrs incubation with the opposite being remarked when increasing the duration of this step. This could be due to the thermal degradation of the biomass sample, over this longer period, inhibiting the action of the solvent thus, conducing to the worst results during the 18 hrs incubation.

Furthermore, although the best performance can still be credited to the CC:U DES with a 48% residue recovery fraction and a solubility of 25.9 mg of rice straw/g of DES during the 18 hrs incubation, the fact that the CC:AA DES manages similar results, with a 49% residue recovery fraction and a solubility of 25.5 mg of rice straw/g of DES, in less than half the time, during the 8 hrs incubation, makes it more suitable for achieving the sustainability goal of this thesis as the energy expenditure will be considerably inferior.

Nonetheless it will be illustrated in the next section that the usage of the CC:AA DES during 4 hrs can lead to even more benefits in the global process.

3.2.4.2 Recovered Liquid Residue

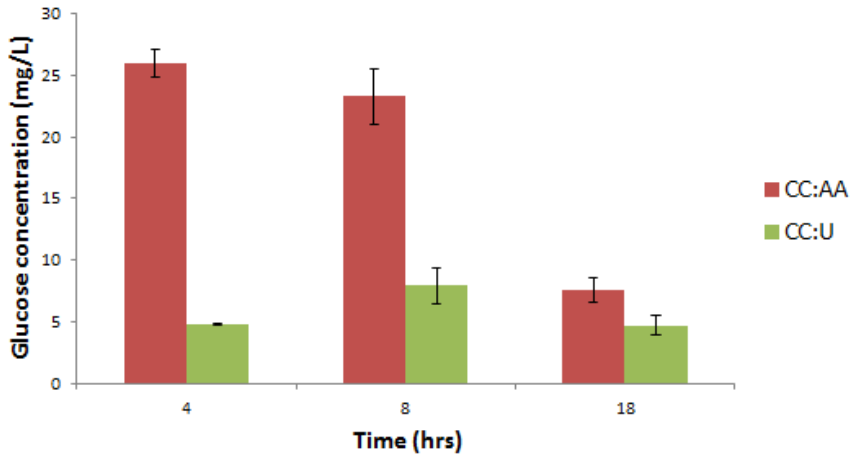


Figure 31 - Glucose yields in the liquid phase residues obtained from the optimization of the fractionation step (+/- 1 S.D.).

From this monomeric sugar analysis, a drastic increase in the glucose concentration of the filtrate can be observed when using the CC:AA DES with a shorter biomass incubation of the lignocellulosic biomass sample. As a matter of fact, an almost fourfold increase in this parameter is remarked between the 18 and 4 hrs step, with this latter attaining a maximal concentration of 26 mg of glucose/L of filtrate.

As for the CC:U DES, a maximum is attained for the 8 hrs incubation, managing a concentration of 7.9 mg of glucose/L of filtrate, slightly higher than the ones obtained with the other durations of this step. The increase

of process efficiency witnessed with this solvent in the passage of the 4 to 8 hrs mark can simply be explained by the longer processing time of the solvent.

However, for the further decline of the glucose content with the longer incubation periods of the assays, both cases may be explained by the degradation of glucose into HMF by the DESs' activity, as is suggested in the literature[104].

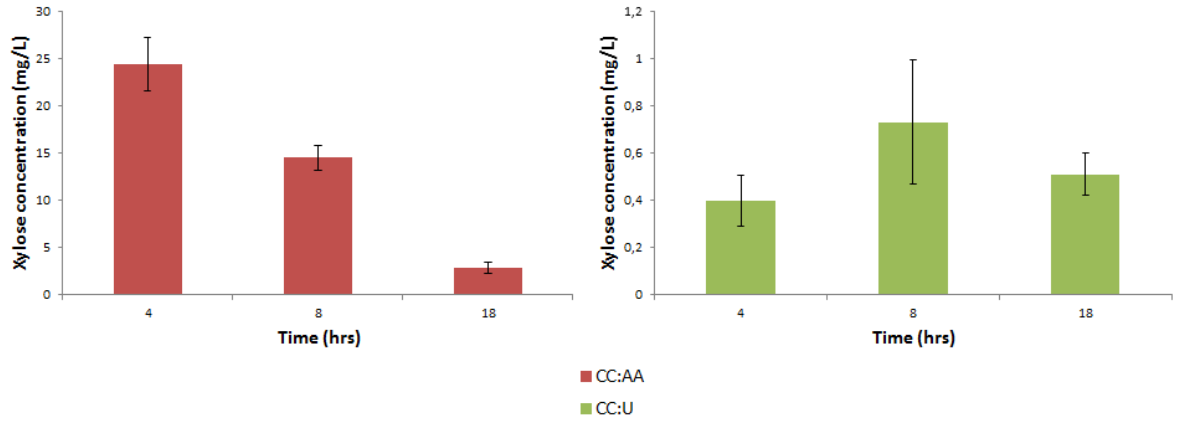


Figure 32 - Xylose yields in the liquid phase residues obtained from the optimization of the fractionation step with both tested DESs (+/- 1 S.D).

In what concerns the xylose concentration of the obtained filtrates, the same exact tendency as remarked before for the glucose content is noted once again for both tested DESs and the same previously mentioned reasons could explain these observations. Nonetheless, it must be mentioned that the product of the xylose degradation would be furfural and not HMF[104].

In conclusion, the choice of the CC:AA DES with a 4 hr biomass incubation leads to the most promising results and it is important to underline that the literature's statement, acknowledging that DESs displaying an acidic nature fare better in these kinds of treatment, is validated[104].

The chromatograms where these results were taken from are also in annex, in appendix D, as well as the calibration curve that was used to determine the glucose and xylose concentrations.

4 Conclusion and Future Perspectives

As the urgent need to find further uses to rice straw, an abundant agricultural residue, is of the utmost importance to avoid its burning across agricultural fields with little usefulness apart from adversely affecting nature's already susceptible fate, this thesis envisaged to tackle this problem through the development of a sustainable process that aimed at the extraction of OZ from rice straw as well as focusing on a biomass treatment to produce products rich in saccharides and polysaccharides that can be fermented and transformed into bioethanol. In order to achieve this objective, a novel class of solvents, named deep eutectic solvents (DESs), was employed to substitute the common volatile organic ones that are usually used in these kinds of processes; their eco-friendlier characteristics grant them a greener nature therefore securing the development of a sustainable process. Furthermore, the use of both hydrophobic and hydrophilic DESs served to showcase the selective design that they can offer as well and thus, extraction and fractionation experiments were carried out with them, according to the literature.

The extraction experiments allowed to infer that no OZ was successfully extracted from the rice straw with the hydrophobic DESs and hexane that was employed for comparison purposes as it is the volatile organic solvent commonly used to extract OZ from rice bran, hence leading to the discovery that the use of rice straw as a source of this compound is not feasible. Nonetheless, this extraction procedure with these novel solvents managed to extract a multitude of other chemicals that have yet to be identified, showing that rice straw has indeed the potential to be a source of added value compounds and as such, valorized.

Additionally, the fractionation experiments, where the high solubility of rice straw in the CC:U DES alongside the selectivity for glucose and xylose of the CC:AA DES were observed, conduced to the finding that the tested DESs are candidates for substituting common volatile organic solvents in the treatment of biomass for creating products that can later be fermented to give bioethanol while once again reinforcing the idea that rice straw can be valorized and its employment useful to face the global energetic crisis. Moreover, the optimization of the fractionation step warranted the use of the CC:AA DES in a 4 hr biomass incubation for achieving the most promising results thus ensuring that the end goal of this project that focused on sustainability was respected. As for the study on the effect of the water fraction in these hydrophilic DESs, it determined that a 10 wt% of water in the used DES aqueous solutions leads to better results over lower concentrations, although a further investigation on the use of higher concentrations of water must yet be performed. The use of severe treatment conditions also justifies an optimization of the temperature at which the process is conducted and it should be mentioned as well that further compositional analysis of the products obtained in these experiments must still be carried out.

And so, to finally answer the proposed problematics formulated at the start of this project:

-Can an efficient process consisting in the usage of DESs to extract OZ from rice straw be developed? If so, how does it compare with current used technologies that are employed to valorize this lignocellulosic residue?

Although an efficient process for extracting OZ from rice straw using DESs could not be developed, the presented thesis has formulated a methodology using hydrophobic DESs for extracting other compounds from rice straw.

-Is there potential to develop an efficient treatment process using the extraction step's residue? If so, how does it compare with other fractionation protocols' results?

The use of hydrophilic DESs in the proposed methodology was applied for directly treating fresh rice straw as the extraction stage was unsuccessful and although an optimization of the developed process is still necessary in order to consider it efficient, the results have shown that it is a promising process for ensuring a valorization route for rice straw with this novel class of solvents.

Thus, for a brighter future to be secured, many changes in today's society must happen and harnessing the total potential of lignocellulosic biomasses is a key factor to turn the tide on the environmental degradation that is witnessed on a global scale. The development of rice straw valorization processes is only a small part of this field of science as the need for new, clean and efficient biomass conversion technologies still persists but it can be said that, besides this presented investigation, the fact that researches are being conducted to harness this biomass's full potential[139][140] seems to point at a hopeful prospect for humanity's fate.

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5 Bibliography

[1] Hobsbawm E *The Age of Revolution: Europe 1789–1848* ed W & N Ltd

[2] Lin D, Hanscom L, Murthy A, Galli A, Evans M, Neill E, Mancini M, Martindill J, Medouar F-Z, Huang S and Wackernagel M 2018 Ecological Footprint Accounting for Countries: Updates and Results of the National Footprint Accounts, 2012–2018 *Resources* **7** 58

[3] Wright L A, Kemp S and Williams I 2011 'Carbon footprinting': towards a universally accepted definition *Carbon Manag.* **2** 61–72

[4] The World Commission on Environment and Development 1992 What is sustainability?

[5] U.S. Environmental Protection Agency 2015 *Sustainability Primer - version 9*

[6] Shaker R R 2015 The spatial distribution of development in Europe and its underlying sustainability correlations *Appl. Geogr.* **63** 304–14

[7] Lucas N, Bienaime C, Belloy C, Queneudec M, Silvestre F and Nava-Saucedo J-E 2008 Polymer biodegradation: Mechanisms and estimation techniques – A review *Chemosphere* **73** 429–42

[8] Toze S and Sidhu J 2011 Biosolids: Human Health Impacts *Encycl. Environ. Heal.* 394–402

[9] Herman D C 2009 Consequences of Biogeochemical Cycles Gone Wild *Environmental Microbiology* (Academic Press) pp 319–33

[10] The United Nations 1997 Kyoto Protocol to the United Nation's Framework Convention on Climate Change

[11] The United Nations 2019 What is the United Nations Framework Convention on Climate Change? | UNFCCC

[12] The United Nations 2019 Introduction | UNFCCC

[13] Liang J 2013 Radiation in the atmosphere *Chemical Modeling for Air Resources* (Academic Press) pp 43–63

[14] Easterbrook D J 2016 Greenhouse Gases *Evidence-Based Climate Science* (Elsevier) pp 163–73

[15] Liang J 2013 Chemical composition of the atmosphere of the Earth *Chemical Modeling for Air Resources* (Academic Press) pp 3–20

[16] Schmithausen A J, Trimborn M and Büscher W 2018 Sources of nitrous oxide and other climate relevant gases on surface area in a dairy free stall barn with solid floor and outside slurry storage *Atmos. Environ.* **178** 41–8

[17] The Intergovernmental Panel On Climate Change *Climate Change 2014 Synthesis Report*

[18] The Intergovernmental Panel On Climate Change 2014 *Global Warming Potential Values*

[19] European Environment Agency 2012 *Greenhouse gas emission trends and projections in Europe 2012: Tracking progress towards Kyoto and 2020 targets*

[20] The United Nations 2019 Assessment of First Phase of Kyoto Protocol Published | UNFCCC

[21] Shishlov I, Morel R and Bellassen V 2016 Compliance of the Parties to the Kyoto Protocol in the first commitment period *Clim. Policy* **16** 768–82

[22] The European Commission Kyoto 1st commitment period (2008–12)

[23] The World Bank Total greenhouse gas emissions (kt of CO2 equivalent) | Data

[24] CNN 2018 Kyoto Protocol Fast Facts

[25] Dessai S 2001 *The climate regime from The Hague to Marrakech: Saving or Sinking the Kyoto Protocol?*

[26] BBC News 2012 UN climate talks extend Kyoto Protocol, promise compensation

[27] The United Nations 2015 Paris Agreement

[28] BBC News 2015 COP21: What does the Paris climate agreement mean for me?

[29] UNFCC 2018 The Paris Agreement | UNFCCC

[30] Victor D G, Akimoto K, Kaya Y, Yamaguchi M, Cullenward D and Hepburn C 2017 Prove Paris was more than paper promises *Nature* **548** 25–7

[31] Huang D, Zhou H and Lin L 2012 Biodiesel: An Alternative to Conventional Fuel *Energy Procedia* **16** 1874–85

[32] Brito Cruz C H, Souza G M and Barbosa Cortez L A 2014 Biofuels for Transport *Future Energy* (Elsevier) pp 215–44

[33] U.S. Environmental Protection Agency 2016 State CO2 Emissions from Fossil Fuel Combustion

[34] The World Bank CO2 emissions (kt) | Data

[35] The World Bank CO2 emissions from gaseous fuel consumption (kt) | Data

[36] The World Bank CO2 emissions from liquid fuel consumption (kt) | Data

[37] The World Bank CO2 emissions from solid fuel consumption (kt) | Data

[38] Doble M, Kruthiventi A K, Doble M and Kruthiventi A K 2007 Alternate Energy Sources *Green Chemistry and Engineering* (Academic Press) pp 171–92

[39] National Biodiesel Board Biodiesel Basics

[40] Ellabban O, Abu-Rub H and Blaabjerg F 2014 Renewable energy resources: Current status, future prospects and their enabling technology *Renew. Sustain. Energy Rev.* **39** 748–64

[41] Clark J H, Luque R and Matharu A S 2012 Green Chemistry, Biofuels, and Biorefinery *Annu. Rev. Chem. Biomol. Eng.* **3** 183–207

[42] O'Callaghan K 2016 Technologies for the utilisation of biogenic waste in the bioeconomy *Food Chem.* **198** 2–11

[43] del Castillo-Romo A Á, Morales-Rodriguez R and Román-Martínez A 2016 Multi-objective optimization for the biotechnological conversion of lingocellulosic biomass to value-added products *Comput. Aided Chem. Eng.* **38** 1515–20

[44] U.S. Environmental Protection Agency Green Chemistry

[45] Anastas P T and Warner J C 1998 *Green chemistry : theory and practice* (Oxford University Press)

[46] European Commission REACH - Chemicals - Environment

[47] European Commission The RoHS Directive

[48] Fernando S, Adhikari S, Chandrapal C and Murali N 2006 Biorefineries: Current Status,

Challenges, and Future Direction *Energy & Fuels* **20** 1727–37

[49] Mandal S, Kunhikrishnan A, Bolan N S, Wijesekara H and Naidu R 2016 Application of Biochar Produced From Biowaste Materials for Environmental Protection and Sustainable Agriculture Production *Environmental Materials and Waste* (Academic Press) pp 73–89

[50] Barrer R M 1943 The viscosity of pure liquids. II. Polymerised ionic melts *Trans. Faraday Soc.* **39** 59

[51] van Osch D J G P, Parmentier D, Dietz C H J T, van den Bruinhorst A, Tuinier R and Kroon M C 2016 Removal of alkali and transition metal ions from water with hydrophobic deep eutectic solvents *Chem. Commun.* **52** 11987–90

[52] Chen L, Mullen G E, Le Roch M, Cassity C G, Gouault N, Fadamiro H Y, Barletta R E, O'Brien R A, Sykora R E, Stenson A C, West K N, Horne H E, Hendrich J M, Xiang K R and Davis J H 2014 On the Formation of a Protic Ionic Liquid in Nature *Angew. Chemie Int. Ed.* **53** 11762–5

[53] Francisco M, González A S B, García de Dios S L, Weggemans W and Kroon M C 2013 Comparison of a low transition temperature mixture (LTTM) formed by lactic acid and choline chloride with choline lactate ionic liquid and the choline chloride salt: physical properties and vapour–liquid equilibria of mixtures containing water and ethanol *RSC Adv.* **3** 23553

[54] Rajiv K 2013 Removal of Surface Contaminants Using Ionic Liquids *Developments in Surface Contamination and Cleaning* (William Andrew Publishing) pp 1–63

[55] Smith E L, Abbott A P and Ryder K S 2014 Deep Eutectic Solvents (DESs) and Their Applications *Chem. Rev.* **114** 11060–82

[56] Florindo C, Lima F, Ribeiro B D and Marrucho I M 2019 Deep eutectic solvents: overcoming 21st century challenges *Curr. Opin. Green Sustain. Chem.* **18** 31–6

[57] Lynam J G, Kumar N and Wong M J 2017 Deep eutectic solvents' ability to solubilize lignin, cellulose, and hemicellulose; thermal stability; and density *Bioresour. Technol.* **238** 684–9

[58] Abbott A P, Capper G, Davies D L, Munro H L, Rasheed R K and Tambyrajah V 2001 Preparation of novel, moisture-stable, Lewis-acidic ionic liquids containing quaternary ammonium salts with functional side chains *Chem. Commun.* 2010–1

[59] Francisco M, van den Bruinhorst A and Kroon M C 2012 New natural and renewable low transition temperature mixtures (LTTMs): screening as solvents for lignocellulosic biomass processing *Green Chem.* **14** 2153

[60] Choi Y H, van Spronsen J, Dai Y, Verberne M, Hollmann F, Arends I W C E, Witkamp G-J and Verpoorte R 2011 Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology? *PLANT Physiol.* **156** 1701–5

[61] Cunha S C and Fernandes J O 2018 Extraction techniques with deep eutectic solvents *TrAC Trends Anal. Chem.* **105** 225–39

[62] Florindo C, Branco L C and Marrucho I M 2019 Quest for Green-Solvent Design: From Hydrophilic to Hydrophobic (Deep) Eutectic Solvents *ChemSusChem* **12** 1549–59

[63] van Osch D J G P, Zubeir L F, van den Bruinhorst A, Rocha M A A and Kroon M C 2015 Hydrophobic deep eutectic solvents as water-immiscible extractants *Green Chem.* **17** 4518–21

[64] Liu X, Xu D, Diao B, Zhang L, Gao J, Liu D and Wang Y 2019 Choline chloride based deep eutectic solvents selection and liquid-liquid equilibrium for separation of dimethyl carbonate and ethanol *J. Mol. Liq.* **275** 347–53

[65] Zeisel S H and da Costa K-A 2009 Choline: an essential nutrient for public health *Nutr. Rev.* **67** 615–23

[66] Dzoyem J P and Eloff J N 2014 Biochemical Parameters in Toxicological Studies in Africa: Significance, Principle of Methods, Data Interpretation, and Use in Plant Screenings *Toxicological Survey of African Medicinal Plants* (Elsevier) pp 659–715

[67] Lewis S M, Ullrey D E, Barnard D E and Knapka J J 2006 Nutrition *The Laboratory Rat* (Academic Press) pp 219–301

[68] Matsumoto N and Nakazato M 2012 Clinical Application of Ghrelin for Chronic Respiratory Diseases *Methods Enzymol.* **514** 399–407

[69] Beare-Rogers J L, Dieffenbacher A and Holm J V. 2001 *Lexicon of lipid nutrition (IUPAC Technical Report)* vol 73

[70] Environmental Protection Agency 2009 Registration Review Final Decision: Caprylic (Octanoic) Acid

[71] Francisco M, van den Bruinhorst A and Kroon M C 2012 New natural and renewable low transition temperature mixtures (LTTMs): screening as solvents for lignocellulosic biomass processing *Green Chem.* **14** 2153

[72] Paola G 2012 *Six Lectures on the Nature of the Hydrogen Bond Lecture 3*

[73] Kumar A K, Parikh B S and Pravakar M 2016 Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue *Environ. Sci. Pollut. Res.* **23** 9265–75

[74] Consultative Group on International Agricultural Research Rice in Europe

[75] European Commission 2015 *EU Rice Economic Fact Sheet*

[76] Kraehmer H, Thomas C and Vidotto F 2017 Rice Production in Europe *Rice Production Worldwide* (Cham: Springer International Publishing) pp 93–116

[77] PORDATA Superfície - Países Europeus

[78] Eurostat *European Population in 2013*

[79] Schramm R, Abadie A, Hua N, Xu Z and Lima M 2007 Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide. *J. Biol. Eng.* **1** 9

[80] Srikaeo K 2014 Organic Rice Bran Oils in Health *Wheat and Rice in Disease Prevention and Health* (Academic Press) pp 453–65

[81] Ma'ruf A, Pramudono B and Aryanti N 2017 Lignin isolation process from rice husk by alkaline hydrogen peroxide: Lignin and silica extracted p 020013

[82] Borresen E C and Ryan E P 2014 Rice Bran: A Food Ingredient with Global Public Health Opportunities *Wheat and Rice in Disease Prevention and Health* (Academic Press) pp 301–10

[83] Sietske Boschma D, Kees I and Kwant W 2013 *Rice straw and Wheat straw: Potential feedstocks for the Biobased Economy*

[84] Jin S and Chen H 2007 Near-infrared analysis of the chemical composition of rice straw *Ind. Crops Prod.* **26** 207–11

[85] Sindhu R, Binod P, Janu K U, Sukumaran R K and Pandey A 2012 Organosolvent pretreatment and enzymatic hydrolysis of rice straw for the production of bioethanol *World J. Microbiol.*

Biotechnol. **28** 473–83

[86] Hames B, Ruiz R, Scarlata C, Sluiter A, Sluiter J and Templeton D 2008 *Preparation of Samples for Compositional Analysis: Laboratory Analytical Procedure (LAP)*

[87] Sluiter A, Ruiz R, Scarlata C, Sluiter J and Templeton D 2008 *Determination of Extractives in Biomass: Laboratory Analytical Procedure (LAP)*

[88] Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D and Crocker D 2008 *Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP)*

[89] Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D and Wolfe J 2008 *Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples Laboratory Analytical Procedure (LAP)*

[90] Goering H K and Van Soest P J 1970 Analytical procedure *Forage fiber analyses (apparatus, reagents, procedures, and some applications)* (U.S. Agricultural Research Service) p 20

[91] Florindo C, Oliveira M M, Branco L C and Marrucho I M 2017 Carbohydrates-based deep eutectic solvents: Thermophysical properties and rice straw dissolution *J. Mol. Liq.* **247** 441–7

[92] Florindo C, Oliveira F S, Rebelo L P N, Fernandes A M and Marrucho I M 2014 Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids *ACS Sustain. Chem. Eng.* **2** 2416–25

[93] Pandey A and Pandey S 2014 Solvatochromic Probe Behavior within Choline Chloride-Based Deep Eutectic Solvents: Effect of Temperature and Water *J. Phys. Chem. B* **118** 14652–61

[94] Dai Y, Witkamp G-J, Verpoorte R and Choi Y H 2015 Tailoring properties of natural deep eutectic solvents with water to facilitate their applications *Food Chem.* **187** 14–9

[95] Morais E S, Mendonça P V., Coelho J F J, Freire M G, Freire C S R, Coutinho J A P and Silvestre A J D 2018 Deep Eutectic Solvent Aqueous Solutions as Efficient Media for the Solubilization of Hardwood Xylans *ChemSusChem* **11** 753–62

[96] Bevenue A and Williams K T 1956 Rice Analysis, Hemicellulose Components of Rice *J. Agric. Food Chem.* **4** 1014–7

[97] Sun R C, Tomkinson J, Ma P L and Liang S F 2000 Comparative study of hemicelluloses from rice straw by alkali and hydrogen peroxide treatments *Carbohydr. Polym.* **42** 111–22

[98] Rodolfo Vegas, José Luis Alonso, Herminia Domínguez A and Parajó J C 2004 Processing of Rice Husk Autohydrolysis Liquors for Obtaining Food Ingredients *J. Agric. Food Chem* **52** 7311–7

[99] Saleh A M and El-Khordagui L K 1985 Hydrotropic agents: a new definition *Int. J. Pharm.* **24** 231–8

[100] Badwan A A, El-Khordagui L K, Saleh A M and Khalil S A 1982 The solubility of benzodiazepines in sodium salicylate solution and a proposed mechanism for hydrotropic solubilization *Int. J. Pharm.* **13** 67–74

[101] Rub M A, Asiri A M, Azum N and Kabir-ud-Din 2014 Investigation of micellar and phase separation phenomenon of phenothiazine drug promazine hydrochloride with anionic hydrotropes *J. Ind. Eng. Chem.* **20** 2023–34

[102] Coffman R E and Kildsig D O 1996 Effect of Nicotinamide and Urea on the Solubility of Riboflavin in Various Solvents *J. Pharm. Sci.* **85** 951–4

[103] John H, Sharlene W and John F 2006 Breakng the Bologcal Barrers to Cellulosc Ethanol: A Joint Research Agenda

[104] Hou X-D, Feng G-J, Ye M, Huang C-M and Zhang Y 2017 Significantly enhanced enzymatic hydrolysis of rice straw via a high-performance two-stage deep eutectic solvents synergistic pretreatment *Bioresour. Technol.* **238** 139–46

[105] Natural Standard Research Collaboration 2011 *Gamma-Oryzanol*

[106] Rogers E J, Rice S M, Nicolosi R J, Carpenter D R, McClelland C A and Romanczyk L J 1993 Identification and quantitation of γ-oryzanol components and simultaneous assessment of tocols in rice bran oil *J. Am. Oil Chem. Soc.* **70** 301–7

[107] Bergman C J and Xu Z 2003 Genotype and Environment Effects on Tocopherol, Tocotrienol, and γ-Oryzanol Contents of Southern U.S. Rice *Cereal Chem. J.* **80** 446–9

[108] Trinovita E, Chany Saputri F and Mun'im A 2018 Potential Gastroprotective Activity of Rice Bran (Oryza sativa L.) Extracted by Ionic Liquid-Microwave-Assisted Extraction against Ethanol-Induced Acute Gastric Ulcers in Rat Model *Sci. Pharm.* **86** 35

[109] Xu Z and Godber J S 2001 Antioxidant activities of major components of γ-oryzanol from rice bran using a linoleic acid model *J. Am. Oil Chem. Soc.* **78** 645

[110] Akihisa T, Yasukawa K, Yamaura M, Ukiya M, Kimura Y, Shimizu N and Arai K 2000 Triterpene alcohol and sterol ferulates from rice bran and their anti-inflammatory effects. *J. Agric. Food Chem.* **48** 2313–9

[111] Xu Z and Godber J S 1999 Purification and Identification of Components of γ-Oryzanol in Rice Bran Oil† *J. Agric. Food Chem* **47** 2274–728

[112] Chemical Book Gamma-Oryzanol Data Sheet | 11042-64-1

[113] Oryza Oil & Fat Chemical Co. *Gamma-Oryzanol, Food and Cosmetic Ingredient*

[114] Sigma-Aldrich *Gamma-Oryzanol Data Sheet*

[115] Takuo T, Takashi Y and Hiroaki S 2011 Method For Producing γ-Oryzanol-Containing Fat Or Oil

[116] Tyakal N I, Ayappankave V and Narayan R S B 2003 Process for the production of oryzanol enriched fraction from rice bran oil soapstock

[117] Hisashi H and Yasuhiko T 1992 Process for producing rice bran oil

[118] Patel M and Naik S N 2004 Gamma-oryzanol from rice bran oil-A review *J. Sci. Ind. Res.* **63** 569–78

[119] Rohman A 2014 Rice Bran Oil's Role in Health and Cooking *Wheat and Rice in Disease Prevention and Health* (Academic Press) pp 481–90

[120] Heidtmann-Bemvenuti R, Saião Nora N and Badiale-Furlong E 2012 Extraction of γ-Oryzanol from Rice Bran *Ciênc. agrotec.* **36** 665–73

[121] Oliveira R, Oliveira V, Aracava K K and Rodrigues C E da C 2012 Effects of the extraction conditions on the yield and composition of rice bran oil extracted with ethanol—A response surface approach *Food Bioprod. Process.* **90** 22–31

[122] Kamimura J A A M, Aracava K K and Rodrigues C E C 2017 Experimental data and modeling of rice bran oil extraction kinetics using ethanol as solvent *Sep. Sci. Technol.* **52** 1921–8

[123] Chen M-H and Bergman C J 2005 A rapid procedure for analysing rice bran tocopherol,

tocotrienol and γ-oryzanol contents *J. Food Compos. Anal.* **18** 139–51

[124] Perkins E G 1995 Composition of Soybeans and Soybean Products *Practical Handbook of Soybean Processing and Utilization* (AOCS Press) pp 9–28

[125] Xu Z and Godber J S 2000 Comparison of supercritical fluid and solvent extraction methods in extracting γ-oryzanol from rice bran *J. Am. Oil Chem. Soc.* **77** 547–51

[126] Srisaipet A and Nuddagul M 2014 Influence of Temperature on Gamma-Oryzanol Stability of Edible Rice Bran Oil during Heating *Int. J. Chem. Eng. Appl.* **5** 303–6

[127] Khuwijitjaru P, Yuenyong T, Pongsawatmanit R and Adachi S 2009 Degradation Kinetics of Gamma-Oryzanol in Antioxidant-Stripped Rice Bran Oil during Thermal Oxidation *J. Oleo Sci.* **58** 491–7

[128] Khuwijitjaru P, Taengtieng N and Changprasit S 2007 *Degradation of Gamma-oryzanol in Rice Bran Oil during Heating : An Analysis Using Derivative UV-spectrophotometry*

[129] Lima F, Gouvenaux J, Branco L C, Silvestre A J D and Marrucho I M 2018 Towards a sulfur clean fuel: Deep extraction of thiophene and dibenzothiophene using polyethylene glycol-based deep eutectic solvents *Fuel* **234** 414–21

[130] Dai Y, Van Spronsen J, Witkamp G-J, Verpoorte R and Choi Y H 2013 *Natural Deep Eutectic Solvents as new potential media for green technology* (Leiden)

[131] Florindo C, Branco L C and Marrucho I M 2017 Development of hydrophobic deep eutectic solvents for extraction of pesticides from aqueous environments *Fluid Phase Equilib.* **448** 135–42

[132] Florindo C, Romero L, Rintoul I, Branco L C and Marrucho I M 2018 From Phase Change Materials to Green Solvents: Hydrophobic Low Viscous Fatty Acid–Based Deep Eutectic Solvents *ACS Sustain. Chem. Eng.* **6** 3888–95

[133] Tetsuya I, Hidemi A, Hiroyasu A and Masao T 1985 Cycloartenyl ferrulate-cyclodextrin complex

[134] Bucci R, Magrì A D, Magrì A L and Marini F 2003 Comparison of three spectrophotometric methods for the determination of γ-oryzanol in rice bran oil *Anal. Bioanal. Chem.* **375** 1254–9

[135] Xiao B, Sun X F and Sun R 2001 Extraction and characterization of lipophilic extractives from rice straw: Chemical compostion *J. Wood Chem. Technol.* **21** 397–411

[136] Zhang C-W, Xia S-Q and Ma P-S 2016 Facile pretreatment of lignocellulosic biomass using deep eutectic solvents *Bioresour. Technol.* **219** 1–5

[137] de Faria E, do Carmo R, Cláudio A F, Freire C, Freire M and Silvestre A 2017 Deep Eutectic Solvents as Efficient Media for the Extraction and Recovery of Cynaropicrin from Cynara cardunculus L. Leaves *Int. J. Mol. Sci.* **18** 2276

[138] Chen W-H, Lin T-S, Guo G-L and Huang W-S 2012 Ethanol production from rice straw hydrolysates by Pichia stipitis *Energy Procedia* **14** 1261–6

[139] Ahmad A, Norfahana A-T and Hamidah Mohd-Setapar S 2018 Removal of silica from rice straw by using alkaline hydrogen peroxide solution in a fixed bed column *J. Mater. Environ. Sci.* **9** 864–972

[140] Zhang Y, Zhang G, Wei M, Gao Z, Tian M and He F 2019 Comparisons of Acid and Water Solubilities of Rice Straw Ash Together with Its Major Ash-Forming Elements at Different Ashing Temperatures: An Experimental Study *Sustainability* **11** 1–18

6 Annex

6.1 Appendix A

Table 14 - Literature summary listing the potential lignocellulosic biomass resources contemplated for this thesis.

Article	Biomass	Solvent	Conditions	Efficiency
Biomass pretreatment using deep eutectic solvents from lignin derived phenols, Green Chem, 2018, 20, 809	Switchgrass	ChCl + HBA, CAT, VAN, PCA	Pre-treatment (lignin extraction): 160°C, 3 hrs	Lignin removal: PCA (60.8%) VAN (52.5%) CAT (49%) HBA (0.4%) Xylan removal: PCA (70.7%) VAN (49.6%) CAT (43.2%) HBA (28.6%) Xylose yields: CAT (42.4%) VAN (35.3%) PCA (28.8%) HBA (19.2%)
Carbohydrates-based deep eutectic solvents: Thermophysical properties and rice straw dissolution, Journal of Molecular Liquids 247 (2017) 441–447	Rice straw	3 HBA: ChCl, AceChCl, BDMACl 5 HBD (Dextro) : Xylose, Mannose, FRU, Glucose, Ribose	Dissolution: 120°C, for at least 24 hrs until no turbidity is noticeable, 400 rpm	Best result: ChCl + Fru 6.5 mg/g
Deep Eutectic Solvent Aqueous Solutions as Efficient Media for the Solubilization of Hardwood Xylans, ChemPubSoc 2018 Feb 22;11(4):753-762	Xylan beechwood E.Globulus wood sawdust	1:2 1)ChCl + AA 2)ChCl + U	Dissolution: 800 rpm Overnight (24 hrs) 90°C S/L ratio: 0.1 0.04 80°C 70°C	1)62 mg/g (65 wt%H2O) 2)304 mg/g (50 wt% H2O) 2)3.1% (50 wt%H2O) 2)14.8% (50 wt%H2O) 2)328 mg/g (33.3 wt% H2O) 2)301 mg/g (20 wt% H2O)

Deep Eutectic Solvents as Efficient Media for the Extraction and Recovery of Cynaropicrin from Cynara cardunculus L. Leaves, Int. J. Mol. Sci. 2017, 18, 2276	Cardoon or wild artichoke; *C. cardunculus* L	Carboxylic acids (But, Hex, Oct, Dec) + Quaternary ammonium salts (N4444/N2222)Cl/Br + Carb.Ac. alone	25°C S/L = 1/10 120 min Optimization: T; 25, 35, 45°C S/L= 1:10 up to 1:50 t: 30 to 1440 min 0-100 wt% H2O	Best result: 6.20 wt% Dec. Ac. + [N4444]Cl 25°C , 60 min, 1:(30-50), 70 wt% H2O
Deep Eutectic Solvents Synthesis, Characterization and Applications in Pretreatment of Lignocellulosic Biomass (Thesis from South Dakota State University)	Prairie cordgrass (PCG) Switchgrass (SWG)	ChCl+U+Glycerol 2/3/1 2/1/3	Optimized conditions: 24 hrs 120°C	Lignin solubility: 14.25 %wt Lignin extraction: 16.6 wt% of PCG 18.7 wt% of SWG Hemicellulose solubility: 0.42 wt%
Deep eutectic solvents' ability to solubilize lignin, cellulose, and hemicellulose; thermal stability; and density, Bioresource Technology, Volume 238, August 2017, Pages 684-689	(Loblolly pine) Alkali lignin Xylan beechwood Medium fibrous celulose	ChCl + Formic A. / LA / AA LA + Betaine / P	60°C 20 min Orbital shaker	Most promising result: LA + ChCl (10:1)
Facile pretreatment of lignocellulosic biomass using deep eutectic solvents, Bioresource Technology, Volume 219, November 2016, Pages 1-5	Corncob	ChCl + Mono and dicarboxylic ac. / Polyalcohol	Optimized conditions: 90°C 24 hrs	Most promising result: Ehtylene glycol + ChCl (2:1)
Significantly enhanced enzymatic hydrolysis of rice straw via a highperformance two-stage deep eutectic solvents synergistic pretreatment, Bioresource Technology, Volume 238, August 2017, Pages 139-146	Rice straw	MA + P ChCl + OA ChCl + U	120°C 4 hrs 5 wt% solids loading	Most promising result: ChCl + U 1:2 (Solubility of pure compounds) ChCl + OA 2:1 (Fractionation)

New natural and renewable low transition temperature mixtures (LTTMs): screening as solvents for lignocellulosic biomass processing, GreenChem, 2012, 14, 2153–2157	Wheat straw + Lignin/Starch/Cellulose	OA dihydrate/ MA/ LA + HBA	60-100^{o}C 24 hrs	Most promising results: Solubility: MA:P (1:3) Better fractionation between lignin and starch/cellulose : LA:Betaine (2:1)
«Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue», Environ Sci Pollut Res Int. 2016 May;23(10):9265-75	Rice straw	LA + ChCl/Betaine	60^{o}C 12 hrs	Most promising result: 68.1 mg Lignin/g biomass Ac.Lactic + ChCl (5:1)
Using a low melting solvent mixture to extract value from wood biomass, Scientific Reports volume 6, Article number: 32420 (2016)	Sawdust made from softwood	ChCl + Boric acid	95^{o}C Overnight (24 hrs)	Best result: Sawdust with granulometry <0.15mm: 49.5% of total lignin dissolved (No water addition)
Ionic liquids and deep eutectic solvents for lignocellulosic biomass fractionation, *Phys.Chem.Chem.Phys.*, 2017, 19, 2636	Article compilation: MCC 3.12 *10^{5} Da MCC	Article compilation	100^{o}C 10 hrs Microwave 80^{o}C 2 hrs Ultrasonication 80^{o}C 1 hr 110^{o}C	Cellulose dissolution in DESs best results: U + ChCl 2:1 Thiourea + ChCl 2:1 Cellulose dissolution in ILs: CH3COO- + C4mim/C1OC2mim

	Eucalyptus PHK-dissolving pulp		90°C	58/56 g/mol Acetic acid + 1-ethyl-3,ethylimidazol-2-ylidene carbene 18 wt%
				Lignin dissolution in ILs:
	Kraft lignin		90°C	CH3COO- + Py / C1mim / Pyrr >50 wt%
				Lignin dissolution in DES:
			100°C	MA:P (1:3) 14.90 wt% (also manages to solubilize 0.78 wt% cellulose)
			60°C	LA:ChCl (10:1) 11.82 wt%
				Most promising result in lignin extractability with DES:
			150°C	IC (7:3) 88.3 wt%
			60°C	LA:ChCl (5:1) 60 wt%
				Best result in saponin extraction: C3mim + Br
Natural Deep Eutectic Solvents as a New Extraction Media for Phenolic Metabolites in Carthamus tinctorius L., Anal.Chem., 2013, 85, 6272–6278	Safflower	LA + Glucose P + MA ChCl + Sucrose/Glucose/Sorbitol/ 1,2,propanediol FRU + Glucose + Sucrose	40°C 1 hr	Extraction of cartormin, carthamin, HSYA: MA:P (1:1) + 25 v% H2O

Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (Carthamus tinctorius), Food Chem, Volume 159, 15 September 2014, Pages 116-121	Safflower	5 DESs: P+MA Glycerol+LA Glycerol+ChCl Sucrose+ChCl Xylitol+ChCl	Lamp light over 15-day period -20^{o}C and 4^{o}C Sunlight	Stabilization of carthamin with DES: Sucrose+ChCl Stabilization of cartormin and HSYA with DES: Sucrose+ChCl
Extraction of saponins from sisal (Agave sisalana) and juá (Ziziphus joazeiro) with cholinium-based ionic liquids and deep eutectic solvents, Eur. Food Res. Technol., 2013, 237, 965–975	Juá Sisal	13 ILs Choline + Acetate/ salicylate/ citrate/ lactate/ succinate/ hexanoate/ benzoate/ butyrate/ malonate/ oxalate/ propionate/ phenylacetate 9 DES urea, glycerol, ethylene glycol, acetic acid, propionic acid, lactic acid, malonic acid, oxalic acid and phenylacetic acid + ChCl (2/1)	40^{o}C 200 rpm 24 hrs Raw material/Solvent = 1/5 Co-solvent 50 wt%	Best extraction based on efficiency, selectivity and low cost: 81% ChCl+A.A. + 19% water (for sisal) Optimization: 245 % Efficiency 10.34 %S 50^{o}C 1.5h 1/10 58% ChCl+P.A. + 42% etanol (for juá) Optimization: 170 % Efficiency 18.39 %S 30^{o}C 1.5h 1/20
Ionic Liquids as Additives for Extraction of Saponins and Polyphenols from Mate (Ilex paraguariensis) and Tea (Camellia sinensis), Ind. Eng. Chem. Res., 2013, 52, 12146–12153	Tea Mate	14 ILs: [C4mim]Cl, [C6mim]Cl, [C8mim]Cl, [C2OHmim]Cl, [Amim]Cl, [Bzmim]Cl, [C2mim][dca], [C2min][EtSO4], [C2min][OTf], [Ch][NTf2], [C2mim][Ac], [C2mim][Lac], [Ch][Ac], [Ch][Hex], [C2mim]Cl,	40^{o}C 1400 rpm 2 hrs IL/water = 1/1 (wt) to raw material (tea and mate grinded leaves) 10/1	Saponin extraction: BzmimCl 55% Mate 65% Tea

		[Ch]Cl		ChCl 50% Mate 53% Tea
Pandoraea sp B-6 assists the deep eutectic solvent pretreatment of rice straw via promoting lignin depolymerization, Bioresource Technology Volume 257, June 2018, Pages 62-68	Rice straw	ChCl + LA (1:5)	90°C, 2-8 hrs 120°C, 2-8 hrs 140°C, 2-8 hrs	Efficiency of depolymerization: Lignin's molecular weight goes from 4780 Da to 2412 Da after pretreatment at 140°C, 8hrs

6.2 Appendix B

Table 15 - List of laboratory material and small equipment used during this project.

Laboratory Material /Equipment	Supplier	Model	Application
Syringes	Braun	1 mL, 2 mL, 5 mL, 10 mL	Preparation of DESs and conduction of extraction experiments
Needles	Braun	1,20 x 50 mm 0,80 x 50 mm	Preparation of DESs and conduction of extraction experiments
Syringe filters	Filter-Lab	PTFE, pore size 0,45 µm, diameter 25 mm	Conduction of extraction experiments
Vacuum pump	Edwards	RV3	Drying of DESs' starting materials
Heating magnetic stirrer plaque	1) VELP Scientifica 2) Heidolph	1) Arex Digital 2) MR Hei-Tec	Preparation of DESs and conduction of extraction and fractionation experiments
Weighting Scales	1)Ohaus 2) Mettler 3) Kern	1) AX223M 2) PB403-S 3) ARJ-NM	Preparation of DESs and experimental assays

Centrifuges	1)Hermle 2)Wifug	1) Z323K 2) Labor-50M	Conduction of extraction and fractionation experiments
Centrifuge tubes	1) Thermo Scientific 2) LabBox	1) - 2) CTSP- 050-050	Conduction of extraction and fractionation experiments
Eppendorf tubes	LabBox	1,5 mL	Conduction of fractionation experiments
Paper filters	Prat Dumas	QNDF-070-100 QLPV-185-100	Conduction of fractionation experiments
Büchner filter	-	-	Conduction of fractionation experiments
Magnetic stirring plaque	Variomag	Poly 15	Preparation of DESs
Vortex	Labnet	Vortex Mixer S0200	Preparation of DESs and conduction of extraction experiments
Thermometer	-	-	Conduction of fractionation experiments
Oven	Memmert	Model 500	Preparation of DESs and conduction of extraction and fractionation experiments
Refrigerator	Indesit	-	Storing of materials as well as of extraction and fractionation experiments' residues
Pro pipettes	1)Labnet 2)LabBox	1) BioPette Plus 100-1000 µL 2) Easy 40 Elite 1000-5000 µL	Preparation of DESs
Thermomixer	Eppendorf	Comfort - 5355	Conduction of fractionation experiments

6.3 Appendix C

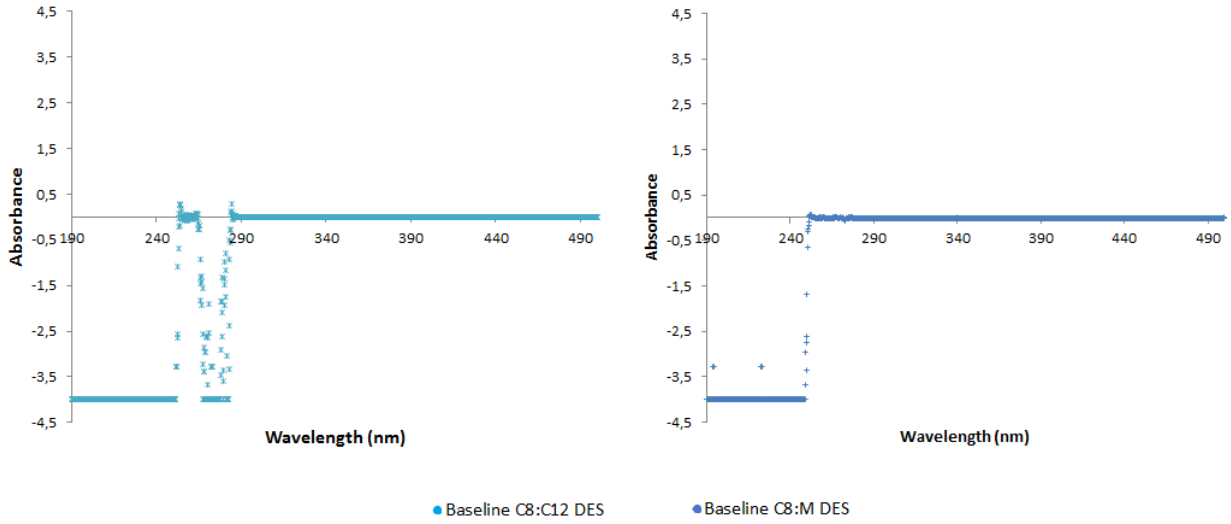

Figure 33 - Spectra of the used baselines in the extraction step's preliminary analyses.

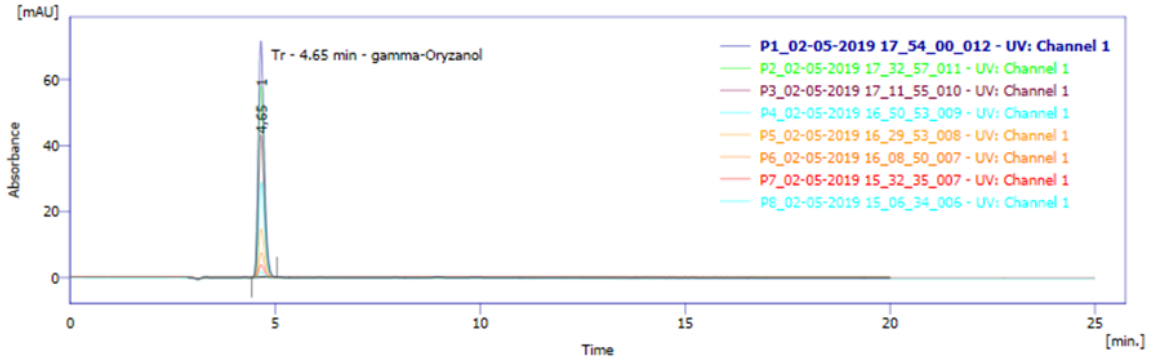

Figure 34 - HPLC chromatogram of the standards composed of OZ in hexane at different concentrations to obtain a calibration curve.

Table 16 - Area of OZ's peaks and respective OZ concentrations to elaborate the calibration curve.

Standard	OZ concentration (mg/L)	Area (UA)
P10	0.15375	3.98
P9	0.3075	11.58
P8	0.615	19.27
P7	1.23	38.39
P6	2.46	74.19
P5	4.92	147.34
P4	9.84	288.26
P3	14.76	430.23
P2	19.68	574.64
P1	24.6	708.67

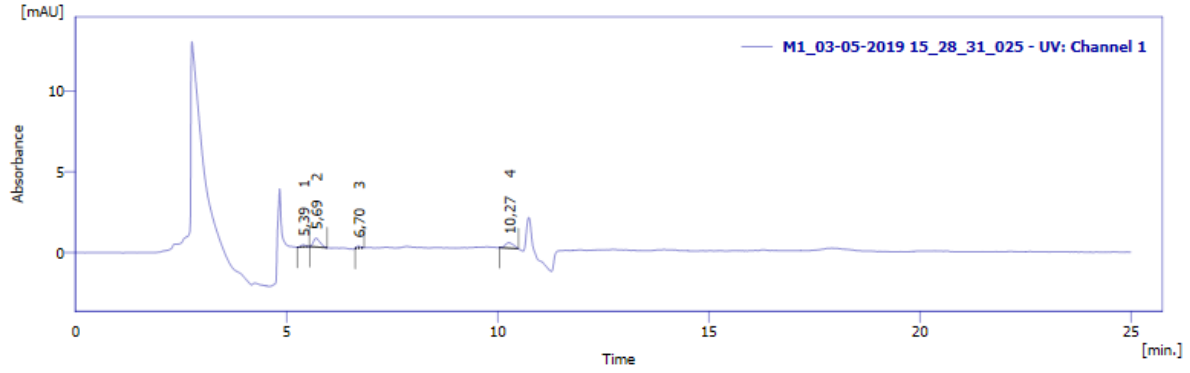

Figure 35 - HPLC chromatogram of an extraction assay with the C8:M DES (1).

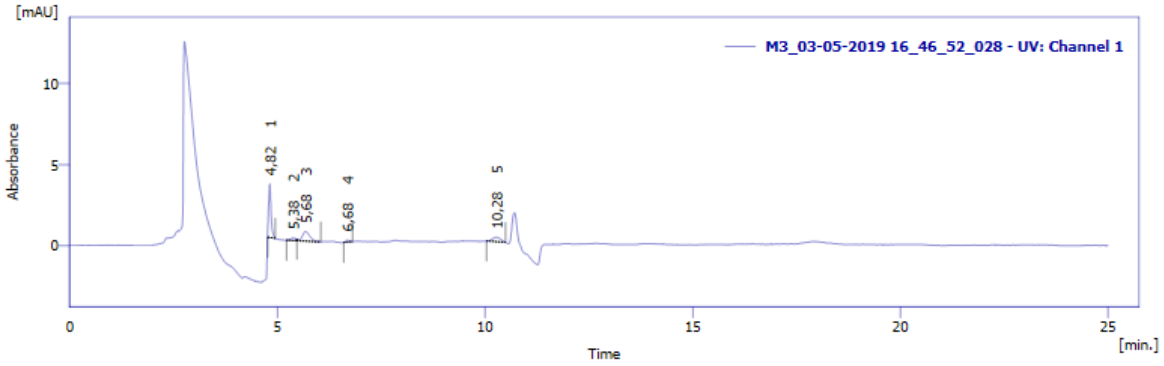

Figure 36 - HPLC chromatogram of an extraction assay with the C8:M DES (2).

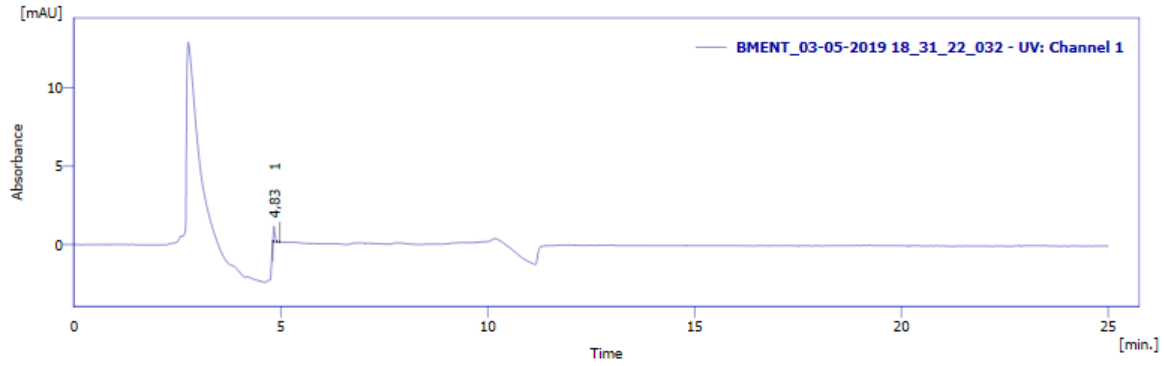

Figure 37 - HPLC chromatogram of the C8:M DES blank OZ extraction assay.

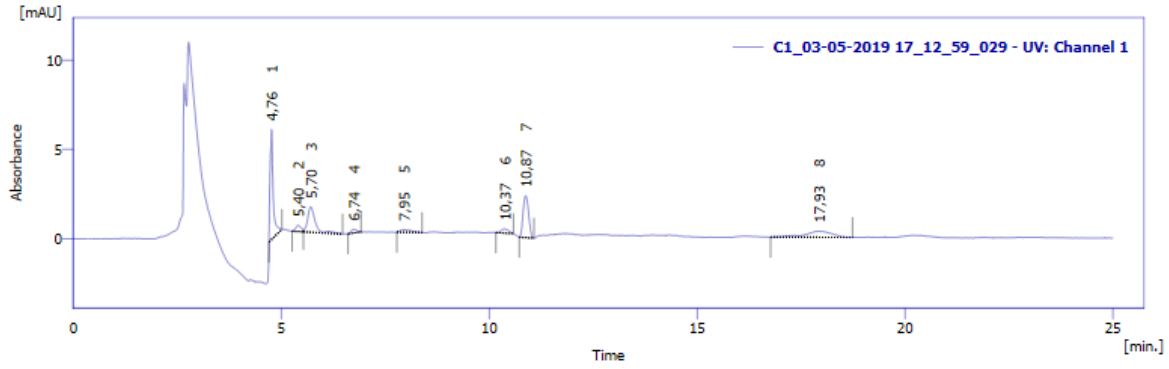

Figure 38 - HPLC chromatogram of an extraction assay with the C8:C12 DES (1).

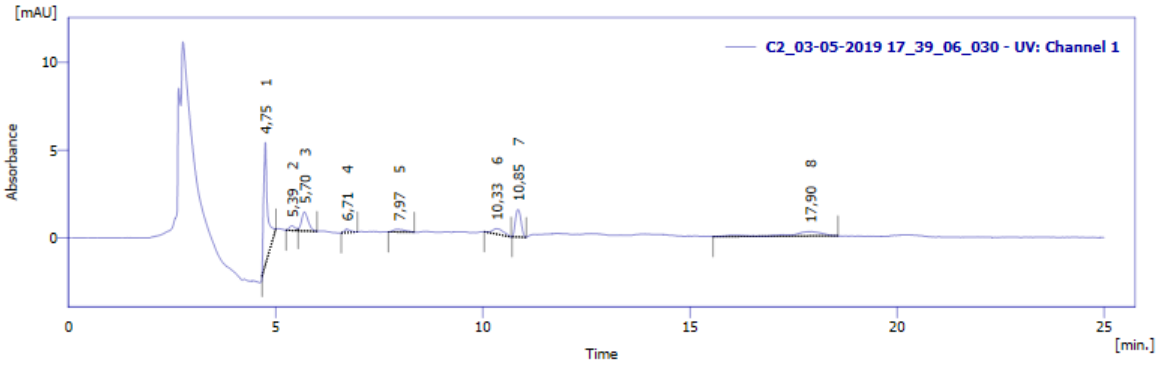

Figure 39 - HPLC chromatogram of an extraction assay with the C8:C12 DES (2).

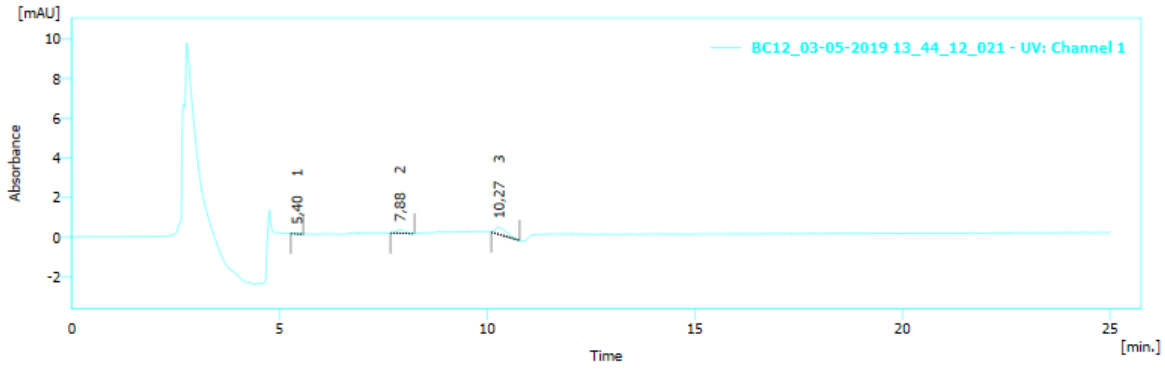

Figure 40 - HPLC chromatogram of the C8:C12 DES blank OZ extraction assay.

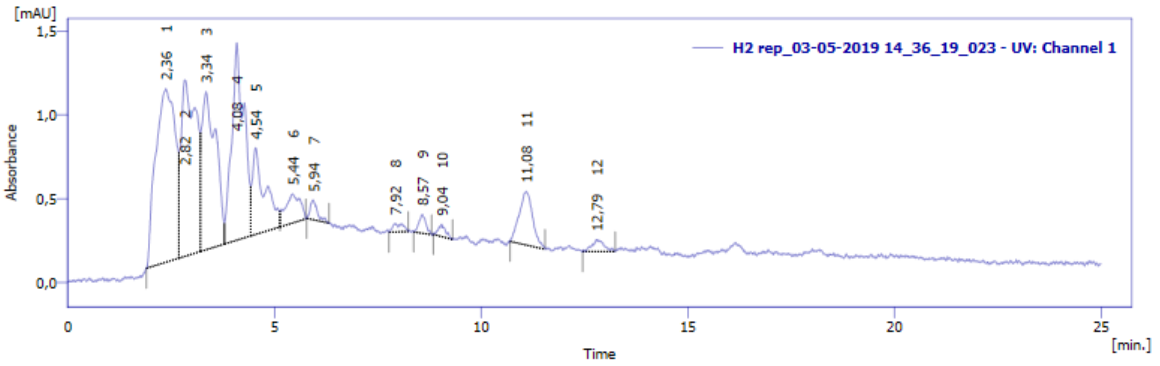

Figure 41 - HPLC chromatogram of an extraction assay with hexane (1).

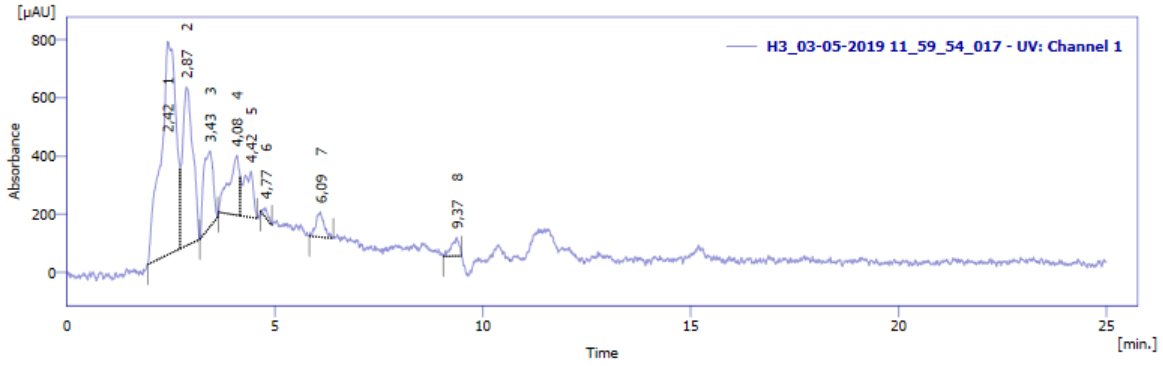

Figure 42 - HPLC chromatogram of an extraction assay with hexane (2).

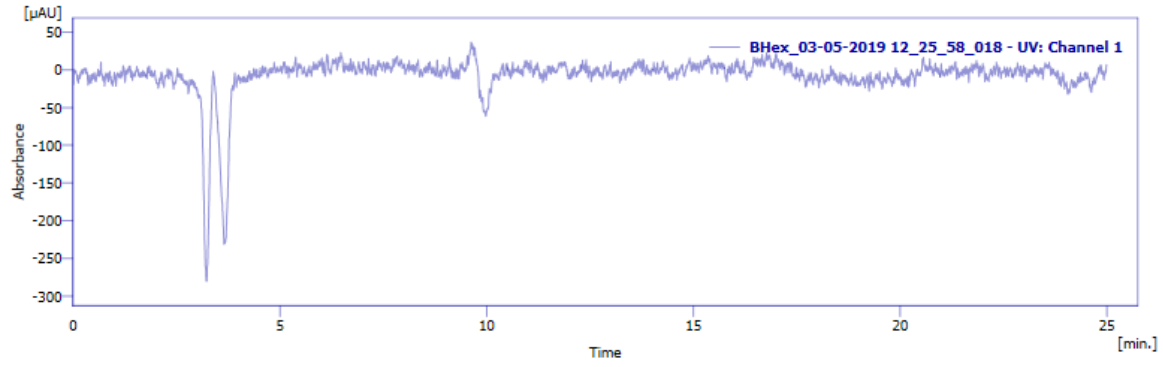

Figure 43 - HPLC chromatogram of the hexane blank OZ extraction assay.

6.4 Appendix D

Table 17 - Area of glucose and xylose peaks and respective concentrations to elaborate the calibration curve for the fractionation.

Standard	Glucose / Xylose Concentration (mg/L)	Glucose Area (UA)	Xylose Area (UA)
1	0	n.a.	n.a.
2	5	10.3104	8.7057
3	10	19.1589	16.5761
4	20	37.9196	33.2574
5	40	71.0465	61.4829
6	60	103.2339	88.4573
7	80	126.1803	107.8989
8	100	147.9199	127.2576

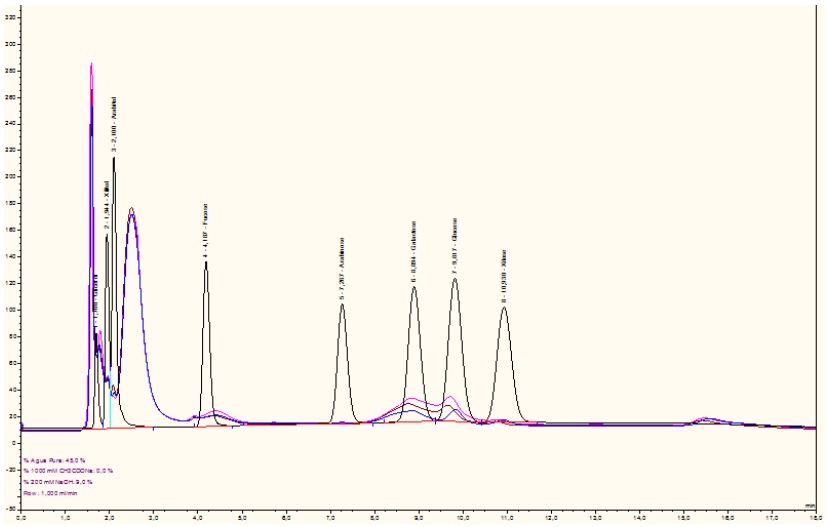

Figure 44 - HPLC chromatogram of the CC:LA DES fractionation assays.

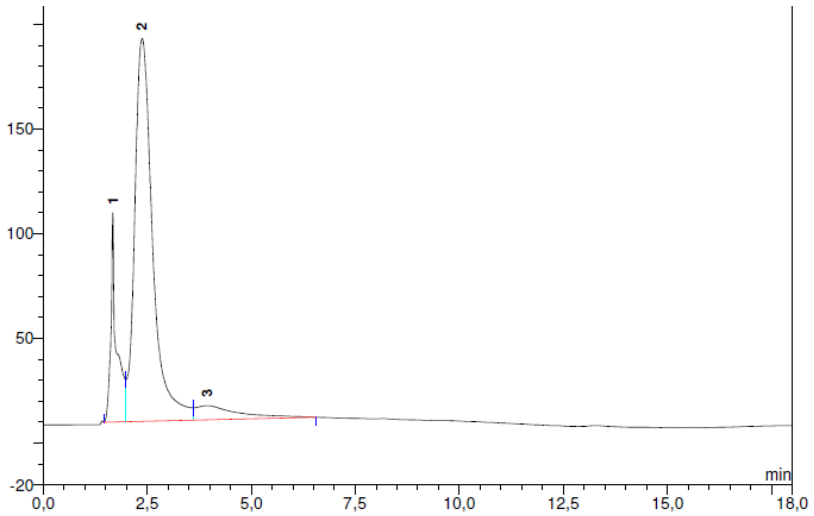

Figure 45 - HPLC chromatogram of the CC:LA DES blank fractionation assay.

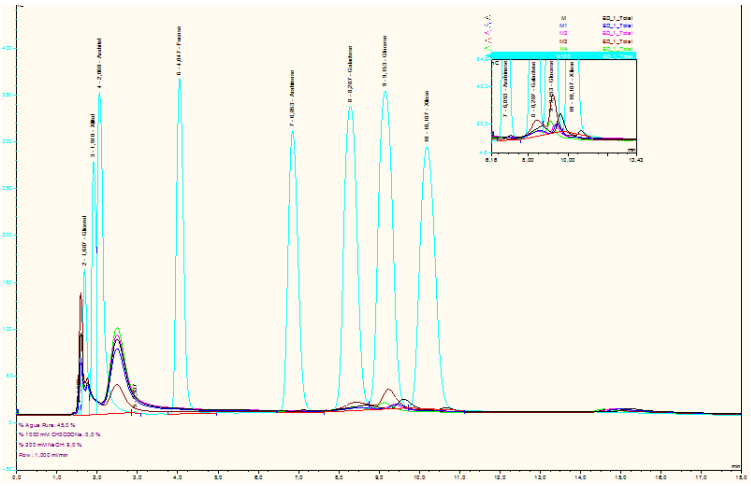

Figure 46 - HPLC chromatogram of the CC:MA DES fractionation assays.

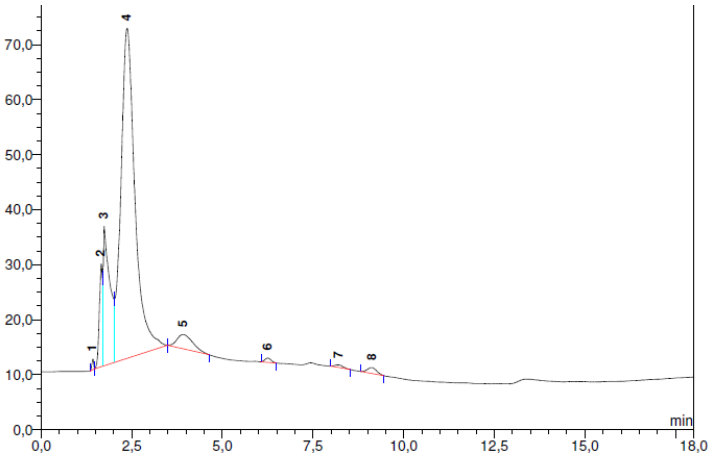

Figure 47 - HPLC chromatogram of the CC:MA DES blank fractionation assay.

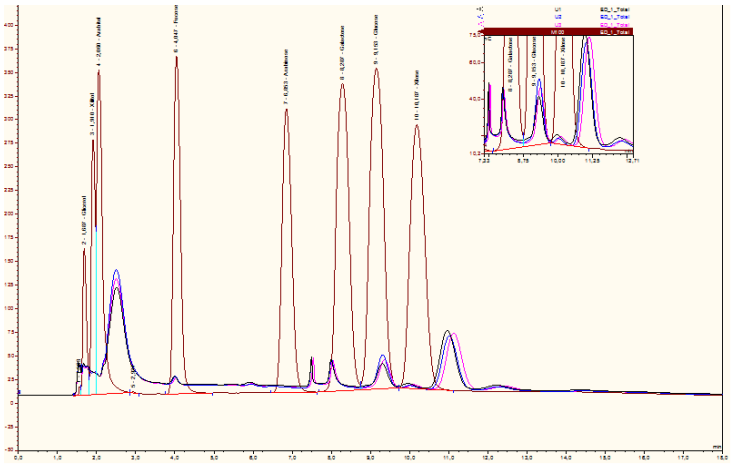

Figure 48 - HPLC chromatogram of the CC:U DES fractionation assays (1).

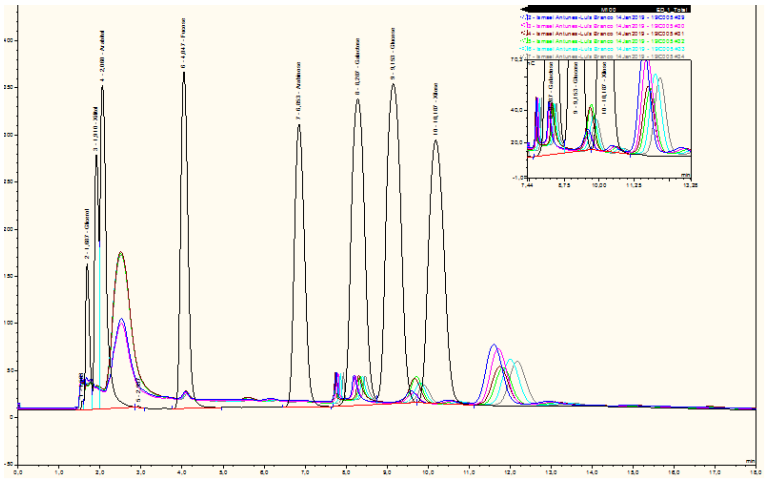

Figure 49 - HPLC chromatogram of the CC:U DES fractionation assays (2).

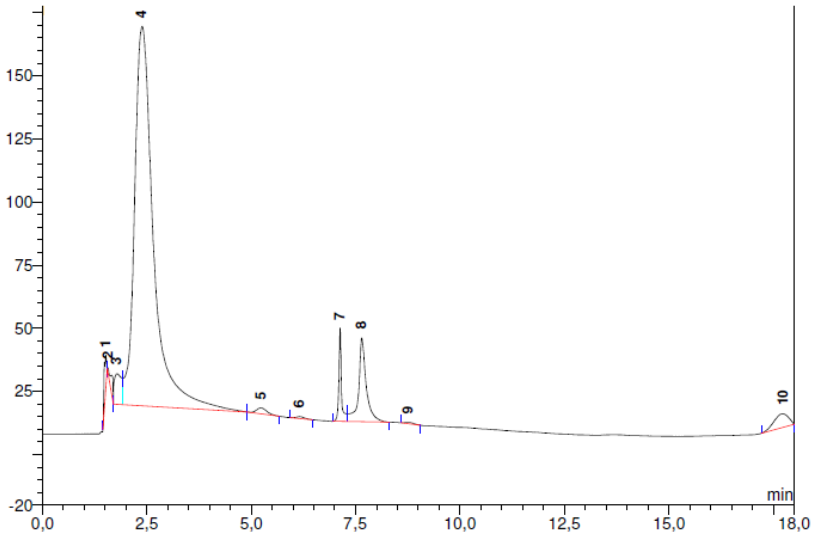

Figure 50 - HPLC chromatogram of the CC:U DES blank fractionation assay.

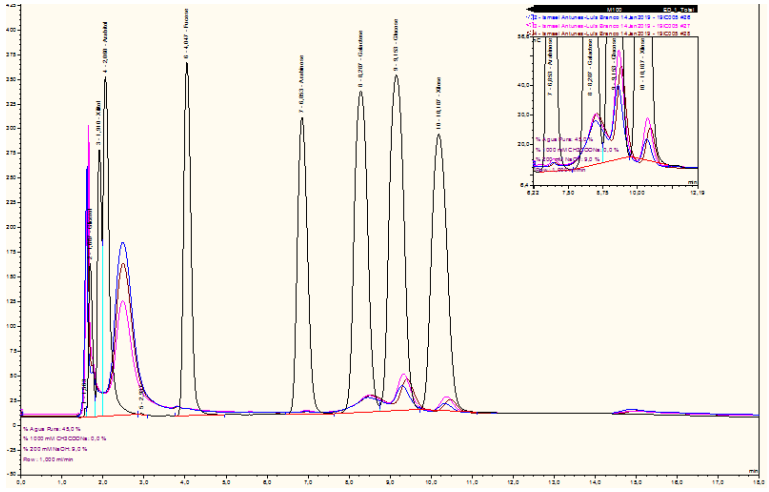

Figure 51 - HPLC chromatogram of the CC:AA DES fractionation assays (1).

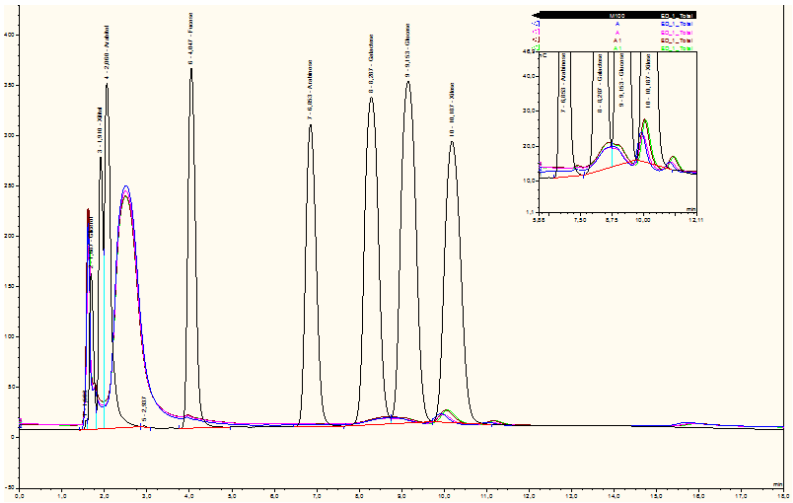

Figure 52 - HPLC chromatogram of the CC:AA DES fractionation assays (2).

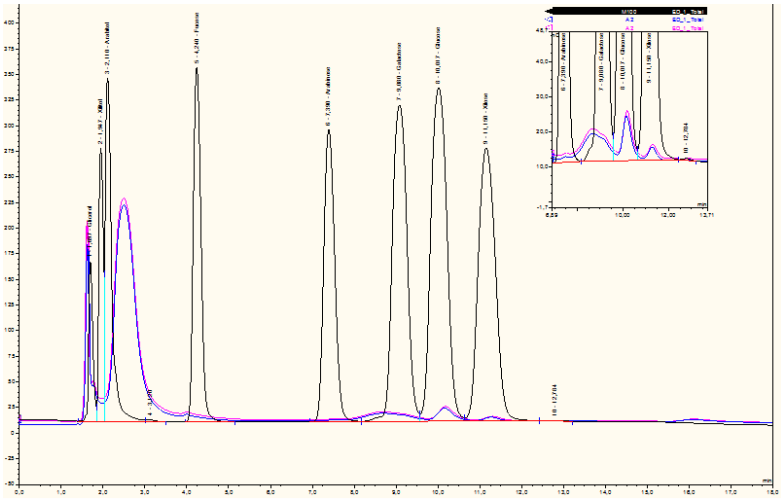

Figure 53 - HPLC chromatogram of the CC:AA DES fractionation assays (3).

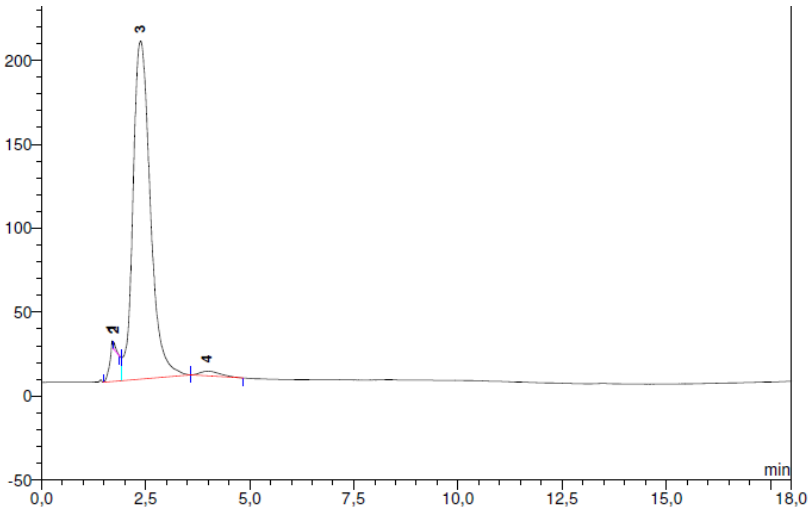

Figure 54 - HPLC chromatogram of the CC:AA DES blank fractionation assay.

Table 18 - Determination of the paper filters' humidity.

Filter	Filter weight (g)	Dry filter weight (g)	Water mass (g)
1	0.3235	0.3210	0.0025
2	0.3329	0.3303	0.0026
3	0.3176	0.3148	0.0028

Table 19 - Area of glucose and xylose peaks and respective concentrations to elaborate the calibration curve for the optimization.

Standard	Glucose / Xylose Concentration (mg/L)	Glucose Area (UA)	Xylose Area (UA)
1	0.5	1.0957	1.0287
2	1	2.1266	2.04
3	2	4.1077	3.9005
4	4	8.517	8.4521
5	8	16.7372	16.398
6	12	24.4486	23.759
7	16	31.2614	30.1073
8	20	39.267	37.3858

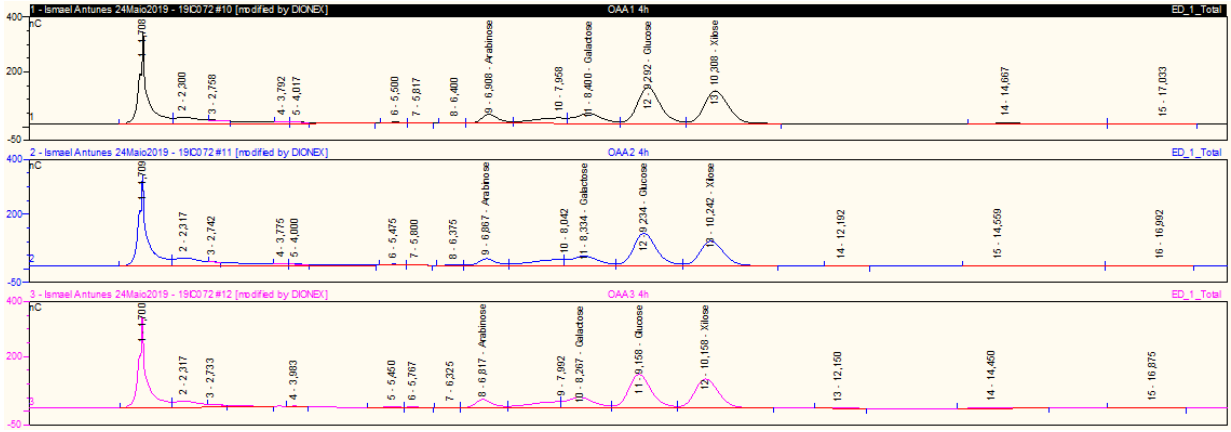

Figure 55 - HPLC chromatograms of the CC:AA DES optimization assays (1).

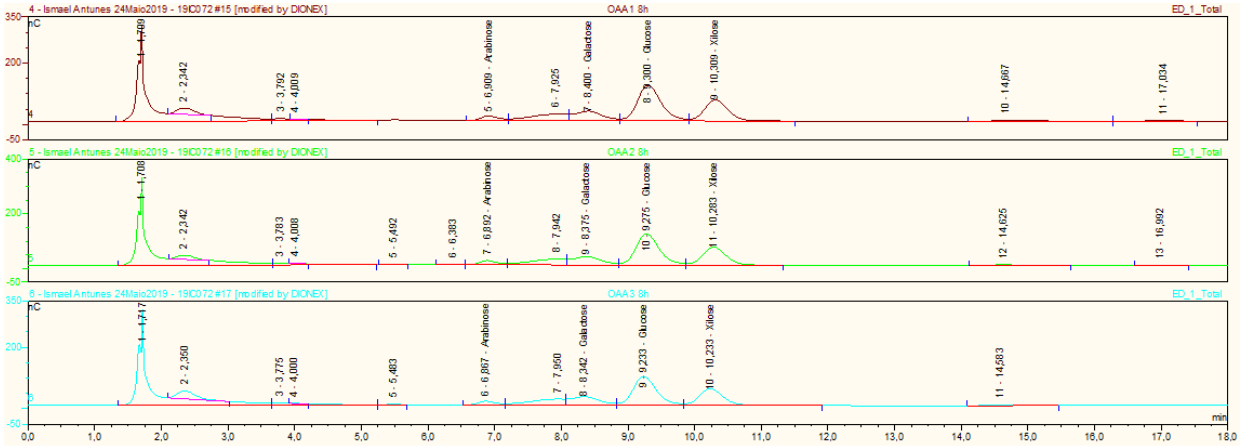

Figure 56 - HPLC chromatograms of the CC:AA DES optimization assays (2).

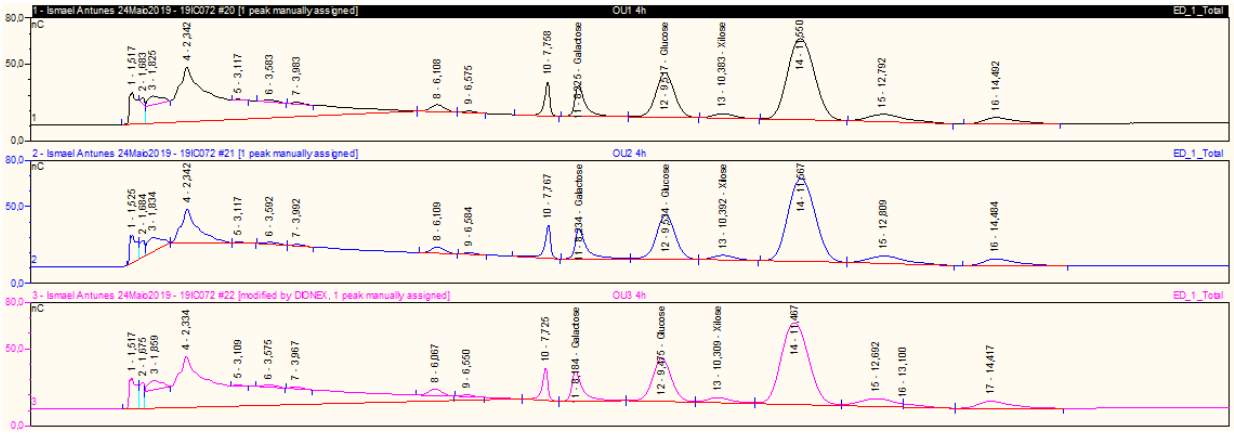

Figure 57 - HPLC chromatograms of the CC:U DES optimization assays (1).

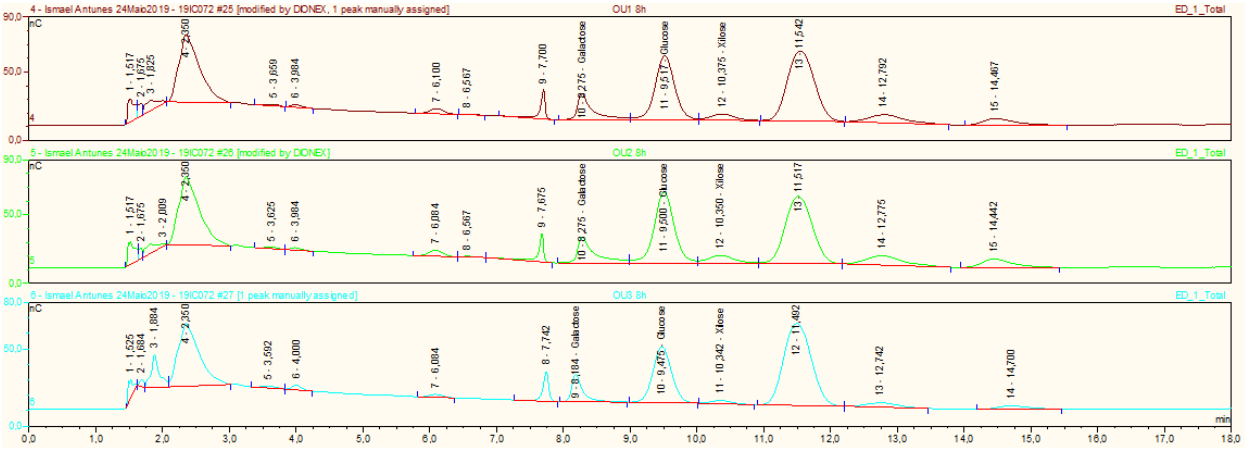

Figure 58 - HPLC chromatograms of the CC:U DES optimization assays (2).