Biomolecules from olive pruning waste in Sierra Mágina

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Keywords: biorefinery, pyrolysis-GCMS, waste reduction; low-carbon olive-growing systems, pathogenic strain, EU policy.

Abstract

The volatility of fossil resources prices, the uncertainty of their long-term availability and the environmental, climatic and societal problems posed by their operation, lead to the imperative of the energy transition, development and use of other alternative and sustainable resources. Acknowledging that indirect land-use change can reduce the greenhouse gas emissions savings, the European Union (EU) has reshaped its biofuel policy. It has a set of sustainability criteria to ensure that the use of biofuels guarantees real carbon savings and protects biodiversity. From a sustainability perspective, biofuels and bioliquids offer indeed both advantages (more secure energy supply, emissions reductions, reduced air pollution, production of high added-value molecules) and risks (monocultures, reduced biodiversity, and even higher emissions through land use change). Approaching economic, environmental and social sustainability at the local level and in an integrated way helps to maximize benefits and minimize risks. This approach has been adopted and described in the present work combining chemical, biological, social and territorial studies. Fungal and social analysis helped to orientate the research towards an attractive two-steps process. High added value molecules detected in the extracts and Py-GCMS analysis indicated the opportunity of an innovative process combining extraction of valuable molecules and pyrolysis in an olive-growing area (Sierra Mágina, Spain) where farmers' margins are decreasing. Further research with a larger partnership will consolidate the results and tackle issues such as the one of logistics arisen by the geographic analysis.

Introduction

Many countries face the challenge of finding reliable, alternative and sustainable fuels and chemicals. Meanwhile, the environmental problematic of biomass waste management remains a challenge while contributing to a sustainable energy supply. Moreover, biofuels produced from biomass needs to be economically viable (from the cultivation of biomass, its collection and processing to the separation of biomolecules/biofuels products). We selected the olive-growing territory of Sierra Magina for the OLIZERO interdisciplinary project. This Mediterranean sloping region, located in Jaén Province (Andalucía), is vulnerable to climate change because of its high level of specialization in olive growing and the burning in loco of pruning waste (dozens of kilos per tree) contributes to greenhouse gases (GHG) emissions [1]. The OLIZERO bio-refinery concept is a model of waste management in agricultural territories. It explores the opportunity to produce simultaneously biomolecules of interest (specialty chemicals, cosmetic applications…) along with biofuels and fertilizers. Indeed, the OLIZERO bio-refinery concept is part of an interdisciplinary thinking which aims to optimize the use of pruning waste available in a specific territory by analyzing local and integrated production of finished and/or intermediate products and higher added-value molecules (from solvents to aroma, flavors and products of medical interest). The final objective is to develop innovative methods for recovering chemicals from the most difficult valuable fractions (lignocellulosic fractions). The project is then about a study of the feasibility of a bio-refinery coupled to existing olive mills. The resource available in the Sierra Mágina County, were initially identified and mapped. Classical Soxhlet extraction and Py-GCMS analysis have been performed on olive tree cuttings from this territory. Most of published studies on the topic are focused on phenolic compounds in olive leaves [2-4]. Indeed, these polyphenols demonstrated to be responsible for their anti-carcinogenic, anti-inflammatory, and antimicrobial proprieties [4]. In the present preliminary study, two classical solvents for such type of extraction have been used for Soxhlet extraction. Besides, fast pyrolysis is an advanced technology to produce biofuels and biomolecules from biomass [5]. In this work Py-GCMS (or analytical pyrolysis) is performed on olive leaves from pruning waste. This technique proved to be a powerful tool to determine the composition of evolved gases from biomass [6]. It provides a picture of what kind of chemicals can be obtained from a pyrolysis process.

Material and methods

European Union's policy: The analysis of the EU's role in setting an overall framework for biofuels development accross Europe is based on a review of the literature produced by the EU institutions (mainly the European Commission and the European Parliament). Additional details and up-dated information have been collected thanks to interviews carried-out at DG ENER, DG REGIO, DG AGRI, DG GROW, DG ENV (respectively in charge of Energy, Regional policy, Agriculture, Internal market and Environment) in 2015 and 2016.

Social perception: The social perception of such biorefinery project has been evaluated together with the changes in pruning practices by a sociological survey. This evaluation relied on a field survey, with 20 in-depth interviews, 40 questionnaires randomly sampled in 8 municipalities, and 2 engaging workshops [7], [Benyei et al. submitted].

Geographic Analysis: We obtained a map of pruning waste from a crossed analysis between field data (pruning waste weight in olive trees of different tree crow sizes) and remote sensing and GIS analysis of the olive tree cover all over the Sierra Mágina region [Jaboeuf; Landolsi 2015 unpublished, 8]. We obtained the spatial criteria of pruning waste management from a GIS analysis between georeferenced 40 farmers' inquiries [7] and geographical data.

Samples: Leaves for soxhlet extraction were obtained from branches brought after the field survey. For Py-GCMS, leaves sampled in different olive groves were used. Finally, a single location has been selected to assess the effect of temperature since the location had no impact on the results.

Reagents: Acetone and diethyl ether were purchased from Sigma Aldrich with 99% and 99.5% purity respectively.

Soxhlet extraction : The samples (3 g) were extracted with 250 ml of each solvent (acetone and diethyl ether) during 16 and 22 h (96 and 132 cycles respectively), refluxing in a Soxhlet apparatus. The extract was then concentrated and chemically analyzed by GCMS analysis device. The solvents were, thereafter, totally evaporated under reduced pressure in a rotary evaporator. The mass of extractives was then measured. The GC methods were the following. For acetone, it began at 303.15K (for 1 min) followed by a heating rate of 5K/min during 12 minutes up to 363.15 K then a heating rate of 8K/min during 23,7 minutes up to 553.15 K and stayed at 553.15K for 1 min. For diethyl ether, the method began at 323.15K (for 1 min) followed by a heating rate of 10K/min during 5 minutes up to 373.15 K then a heating rate of 8K/min during 22,5 minutes up to 553.15 K and stayed at 553.15K for 1 min.

Py-GCMS method : Samples of olive pruning waste (leaves), prepared with reproducible weights of 0.5 +/- 0.1 mg, have been analyzed using analytical pyrolysis (Pyrolyzer PY3030, Frontier Lab) coupled to a gas chromatograph and mass spectrometer (GCMS-QP2010Ultra SHIMADZU) under continuous Helium flow (1.24 ml/min). The GC was equipped with a polar capillary column ZB-1701 (30mx0.25µmx0.25µm) with a (14 %-cyanopropyl-phenyl-86 %-dimethylpolysiloxane) phase. As a preliminary step, 2 samples were analyzed for 3 different locations at 773.15K. Then, two pyrograms were produced from the same sample location at four different temperatures (673.15K, 773.15, 873.15K, and 973.15K). In order to provide selective compound separation, the GC heating method began at 303.15K (for 1 min) followed by a heating rate of 3K/min during 13,3 minutes up to 343.15 K then a heating rate of 8K/min during 26,2 minutes up to 553.15 K and stayed at 553.15K for 1 min. For Py-GCMS and GCMS analysis, the temperatures of the injector and detector were set to 553.15K and 473.15K respectively. The ionization mode on the MS was electron impact. The mass range from m/z equal to 25 up to 600 was scanned and the identification of the compounds relied on the mass spectra library from NIST. The results were relative and qualitative. They were expressed in area percentages of the Total Ion Chromatogram (TIC).

Olive tree endophyte identification. Samples of olive pruning wastes were surface sterilized and deposited onto potato dextrose agar (PDA) Petri. After five days at 27°C, fungal mycelia originating from the samples were collected and inoculated onto fresh PDA plates. After purification, species were identified by sequencing their barecode region (ITS spacer region).

Results

EU Policy

The OLIZERO project takes place against a background characterized both by an increasing share of renewable energies in the European energy mix and by controversies related mainly to social acceptance of these energies (especially in the case of wind energy) and to the carbon footprint of bioenergy.

Against this background, a better mobilization of cellulosic wastes and residues in EU countries (estimated by Searle and Malins [9] as the quantity of each feedstock left over after environmental concerns and existing uses are taken into account) would be relevant. The amount of material left on site under sustainable harvesting practices to protect against soil erosion and soil carbon loss varies among EU Member States but is significant in some areas [9]. Bio-refineries supplied from lignocellulosic biomass and implementing emerging technologies such as pyrolysis process in an olive-growing area would in this context be relevant as it would address at the same time sustainability issues linked to bioenergy and uncertainties of the olive oil sector in Europe.

The EU accounts for almost three quarters of global production of olive oil, the bulk of which being concentrated in four countries (Spain, Italy, Greece, Portugal). Spain (and especially Andalusia) depends heavily on big holdings whereas the sector is more fragmented in other producing countries [10]. The European Commission expects the production to grow significantly in the years to come and the area of irrigated olive grove could expand accordingly between 2011 (681 000 ha) and 2020 (771 000 ha). Olive oil production could reach 1.68 million tons by 2020, the exact figure being subject to climatic conditions. This trend is however disconnected from margins and income indicators that have shown a clear downward trend since 2000. From 2000 to 2009, income in Spanish olive farms has experienced a one-third drop in nominal terms (-38% in family income per work unit) in a context of lower market prices [11]. Thus, harnessing the full value of their production through additional by-products makes sense for farmers, especially in areas such as Andalusia where complementary income sources are scarce.

In its Action Plan adopted in 2012 [12] to support the olive oil sector, the European Commission did acknowledge the overproduction crisis of the sector in Spain but didn't mention the role the sector could play in the energy supply. The stress was instead put on the improvement of quality standards and on better structuration of the supply chain in order to strengthen the bargaining power of producers. Agri-environmental measures have also been advocated in the framework of the second pillar of Common Agricultural Policy (CAP). The CAP has indeed been reshaped for the 2014-2020 programming period and substantial changes have been introduced concerning environmental protection. 30% of direct payments to farmers are now subject to compliance with environmental "greening" measures. Furthermore, environmental protection, including climate change aspects and the production of renewable energy has been strengthened in the Rural Development Policy. In each Member State, 30% of rural development funds have to be spent on measures linked to the environment policy or to climate change mitigation. Hence, funding for innovative solutions aimed at optimizing the use of available residues might be found in the Cohesion and in the Rural Development policies rather than in the framework of the Energy policy.

Social Perception of the project and change in pruning practices

The burning of pruning waste is currently being partly replaced by chipping and composting chips on the soils, a practice with less emission of GHG, but which generates a potential risk of pest contamination [13], mostly unknown by farmers. Along with this transition, local stakeholders showed their interest for OLIZERO project, but made suggestions that the team took into consideration within a multi-actor approach. The first suggestion was to fully integrate the carbon balance of the innovation (for example the cost of the transport of the pruning waste), the second concern was the economic viability of the innovation, and the possible return for the farmers (who invested in machines and energy for pruning waste chipping). Moreover, according to farmers, chipping pruning waste and composting on the ground bring benefits for the soil, avoiding erosion and fertilizing it. For this reason, we introduced the return of biochar to compensate the "loss" of the compost due to the project (chips will be used in the pyrolysis process), as this residue is recognized as a fertilizer [14].

Geographic Analysis

Pruning waste varies depending on the tree age and the pruning frequency. Geographic analysis showed that the valorization of pruning waste will have to deal with the quantity of biennial pruning waste in tons per hectare varying from 1 to 4. The less productive olive-groves are new plantations located in large flat plots. The choice of waste management depends on the distance between a viable road and the grove; above 250 meters, farmers continue burning the pruning residue. The road system and the slope will be major issues for spatial optimization of the wastes collection. Basic data still need to be improved to perform such spatial modeling.

Endophytic fungi Analysis

Analysis of Endophytic fungi present in pruning residues showed that the major species (28%) belonged to the genus *Alternaria*, a group that is known to contain many strains pathogenic to olive trees as previously found in Olive tree from the Baleares [15]. Additional species were found to belong to Pezizomycetes, Dothideomycetes and Sordariomycetes, some of which also contain strains potentially pathogenic to the olive trees. Leaving the pruning materials unattended may therefore facilitate the emergence of opportunistic strains able to infect weakened trees, a condition that may become prominent due to climatic change. Pruning residues should therefore be disposed of in a way that would limit fungal growth. We proposed that in replacement to burning, pyrolysis will be able to dispose in a safe way of pruning residues, in addition to produce high-value-biomolecules and fertilizer.

Biomolecules Production

The analysis of the extractives by GCMS revealed different families of molecules such as acids, fatty acids, terpenes, tannins and fatty alcohols. The detailed composition of the extracts is described in Table 1. These molecules are often present in small amount (approximately 5%wt in the extractive part of lignocellulosic materials). In this work, 10%wt extractives have been recovered using acetone as extraction solvent and 3.3%wt with diethyl ether. More products were also detected from acetone extracts. The obtained extractives have useful applications in various fields such as lubricants, solvents, plastics, surface agents, cosmetics, polymers, resins, soap, detergents, fragrance…

Py-GCMS experimental device enabled to identify the decomposition products of the 3 major polymers in lignocellulose [16] (lignin, cellulose and hemicelluloses). In order to have an overview of the main biomolecules that could be produced via pyrolysis, the samples have been injected in a preheated oven at four different temperatures (673.15, 773.15, 873.15 and 973.15 K). Results are presented in Table 2. The pyrogram at 717.15K showed the highest number of peaks (Figure1). The results showed expected molecules from lignocelluloses degradation with high intensity peaks. Indeed, typical phenolics, aldehydes, ketone and acetic acid were recovered. The applications of these molecules are well known in fields such as resins, solvents, chemicals, aroma etc. Some components such as isosorbide and phytol acetate were more specific to the present feedstock. Isosorbide is famous for its pharmaceutical properties and its use in the polymer industry. Phytol acetate is a flavor and fragrance agent. These molecules were in bold characters in Table 2.

The increase in temperature favored the formation of low molecular weight molecules. The proportion of linear fractionation products increased while the one cyclized compounds decreased. The complementary analysis of both extraction and pyrolysis coupled with GCMS analysis helped to identify biomolecules production potential. These molecules of interest have various potential applications ranging from cosmetics, chemicals, materials to antioxidant precursors synthesis.

Discussion

Bioenergy plays a key role in the European energy transition since it represents two-thirds of Europe's renewable energy supplies. The growth of bioenergy from 2010 to 2015 in absolute terms was as important as the growth of all other renewable sources together (6,2 Mtoe per year) [17]. However, its sustainability and its carbon footprint remain much discussed. Forest biomass has long been perceived as non-controversial since the total volumes of wood available in Europe far exceed the demand. However, the changing patterns and increase in competition over forest resources are not always considered in national forest policies. Conflicts are now common in establishing trade-offs between different demands coming from energy suppliers, pulp-and-paper industry and other industrial sectors [18] even if motives and challenges differ from one case to another [19,20].

The support provided to biofuels has however triggered critics pointing to indirect land use change (ILUC) induced by this policy that could lead to higher global food prices and could offset emissions reductions. While biofuels industry has dismissed concerns related to food production, biofuels sustainability remains controversial as its impact on greenhouse gas emissions (GHG) remains uncertain. The 10% target for renewable energy in the transport sector has mainly benefited to crop-based biofuels, which negative environmental impacts are well documented.

In 2015 the European Commission proposed capping conventional biofuels (to 5%) and promoting advanced biofuels, a proposal endorsed by the Council and by the Parliament (albeit with a 7% capping instead of 5%) [21].

Bioenergy sustainability will remain a key issue in the years to come. The Commission indicated in 2014 in its communication on the EU2030 climate and energy framework [22] that “an improved biomass policy will also be necessary to maximize the resource efficient use of biomass in order to deliver robust and verifiable greenhouse gas savings (...). This should also encompass the sustainable use of land, the sustainable management of forests in line with the EU's forest strategy and address indirect land use effects as with biofuels". While putting forward it its Communication on Energy Union (February 2015) a new Renewable Energy Package to be submitted in 2016-2017, the European Commission made clear that the package would include a new policy for sustainable biomass and biofuels [21]. Hence, the need for solutions that prevent loss of biodiversity and higher emissions through land use change. The olive industry dominated by the EU is particularly sensitive to this challenge and therefore in need for such solutions.

In this work, we proposed a strategy for pruning waste valorization involving local stakeholders. The field study indicated that a favorable solution should emerge from a multi-actor approach integrating the concerns and perceptions of local actors. Farmers' belief that chipping pruning waste and composting on the ground would bring benefits for the soil has been seriously questioned by endophytic fungi analysis. These practices seemed to be risky since the pathologic strains detected might induce trees infection. In coordination with local stakeholders, the team OLIZERO performed preliminary studies. The possibility to develop a two-steps process has been identified. The first step would be an extraction process of high added value molecules considering the quantity and quality of extractives obtained. In the actual plant, green solvents and advanced method such as supercritical extraction would be favoured. Secondly, the residual lignocellulosic biomass would be pyrolyzed in order to produce new biomolecules of interest, biochar and energy. Globally, this biorefinery would be able to produce at the same time olive oil, energy, high added value molecules, biofuels and biochar for the soils. The geographic analysis showed strong spatial heterogeneities which emphasized the importance of logistics and the need for waste collection optimization. Therefore different scenarios for the biorefinery implantation needs to be considered (mobile pyrolysis, plant next to existing olive mills, size, number and positioning…)

The interdisciplinary approach highlighted the challenges that will be faced. The technical and economic feasibility of the process shall be demonstrated in accordance with the collection and plants positioning issues. Involvement of local stakeholders is crucial in order to understand their awareness about the risk of trees infection, their positions towards rural development policies and their opinion about different technologies such as pyrolysis.

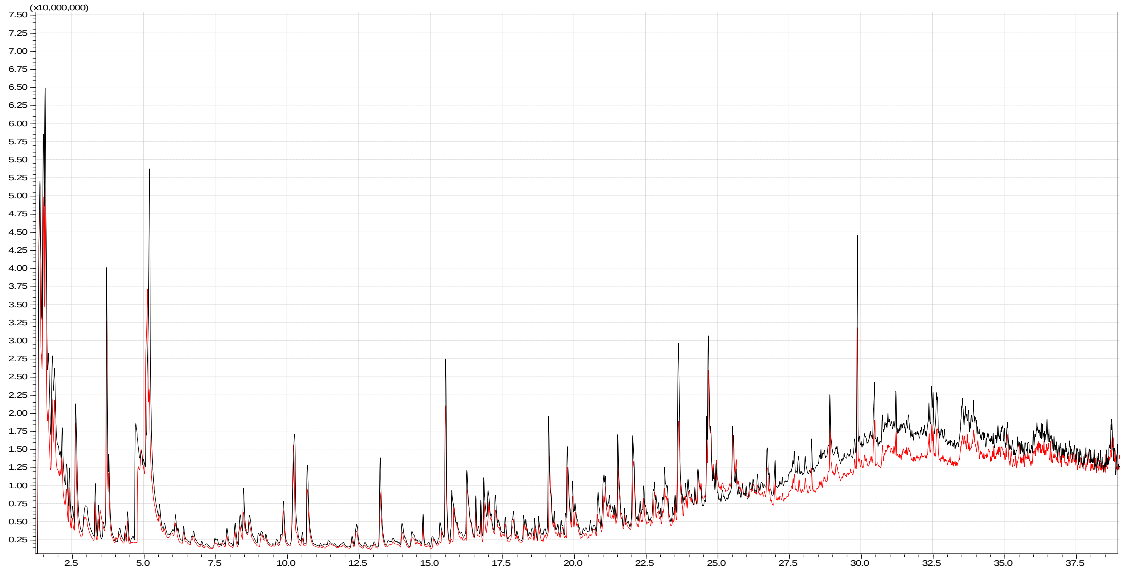
Conclusions

The collaboration between social, biological and chemical engineering sciences brought many advantages: first, we chose a territory with a social demand to improve the waste management, along with a capacity of innovation. This encourages us to improve the agronomical and economic benefits of the proposed innovation. Secondly, the analysis of endophytic fungi showed the potential risks of the changing practices along with the potential benefit of our innovation. Finally, the chemical and Py-GCMS analysis highlighted the opportunity of a two-steps process making possible to extract high added value molecules, produce energy and fertilizer. Further investigations, such as on-going laboratory and pilot scale pyrolysis, are needed to address the technical challenges in the production processes and in the design of appropriate separation technologies. However, knowing that potential molecules of interest could be recovered is encouraging for the future developments of OLIZERO bio-refinery concept. It will be necessary to define the geographic data (workflow, localization, mapping etc.) to plan the availability of the biomass according to harvest periods and obviously physicochemical characteristics to assess the nature of bio-molecules that will be extracted (volumes and material flow). In the prospect of this global study that involved, at Spanish and French levels, several partners, complementary tools and skills, future work will be dedicated to the consolidation of this innovative methodology at UE level by enlarging the partnership to other academics and private companies interested by waste management through alternative biomolecules/fuels production.

Acknowledgments

IdEx Sorbonne Paris Cité and Région Ile-de-France are acknowledged for their financial support.
Pôle image and Coumba Doucouré (Université Paris 13) are acknowledged for their technical support.

Figure 1 : Pyrograms obtained from flash pyrolysis-GCMS at 773,15 K



Tables

Table 1: Major products obtained by GCMS analysis after Soxhlet extraction of olive tree leaves from cuttings at 313.15 K

Acetone solvent	**Diethyl Ether solvent**
Acetic acid	Acetic acid
3-Tetradecanol acetate	Phenol
1-Dodecene	Squalene
1-Tetradecene	Nonacosane
Cycloheptasiloxane, tetradecamethyl-	Butylated Hydroxytoluene
1-Pentadecene	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]-
Phenol, 2,4-bis(1,1-dimethylethyl)-	3,7,11,15-Tetramethyl-2-hexadecen-1-ol*
1-Eicosanol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol*
7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	2-Pentadecanone, 6,10,14-trimethyl-
3,7,11,15-Tetramethyl-2-hexadecen-1-ol*	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol*	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol*	
2-Pentadecanone, 6,10,14-trimethyl-	
Carbamodithioic acid, diethyl-	
Tetradecanoic acid, 12-methyl-, methyl ester	
3-Tetradecyn-1-ol	
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	
Benzoin	
2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4a-methyl-	
Phthalic acid, butyl undecyl ester	
1,2-Butanediol, 1-phenyl-	
4-Hydroxy-3,3,5-trimethyl-6-oxa-bicyclo[3.1.0]hexan-2-one	

*: precise identifications of molecules with large carbon numbers are difficult via MS

Table 2: Major products obtained by Pyrolysis-GCMS analysis of olive tree leaves from cuttings at 4 temperatures

673,15 K	773,15 K	873,15 K	973,15 K
Formic acid	Acetic acid, oxo-	Butanal, 3-hydroxy-	Butanal, 3-hydroxy-
Acetaldehyde	Methyl glyoxal	Acetic acid, anhydride with formic acid	Acetic acid, anhydride with formic acid
Methyl Alcohol	Methyl Alcohol	Methyl Alcohol	1-Pentanol
2,3-Butanedione	1,3-Butadiene, 2-methyl-	(*) Cyclopropane, ethylidene-	1,3-Butadiene, 2-methyl-
2-Butenal	2-Propenal	2-Propenal	1,4-Pentadien-3-ol
Acetic acid	Acetone	1-Hexene	1-Hexene
Oxiranemethanol, (S)-	2,3-Butanedione	.alpha.-Acetobutyrolactone	2-Pentene, 4-methyl-
Propanoic acid, 2-oxo-, methyl ester	2-Butenal	2,4-Hexadien-1-ol	2-Hexen-1-ol, acetate, (Z)-
Furfural	Acetic acid	2-Butenal	1,3,5-Hexatriene, (Z)-
2-Propanone, 1-(acetyloxy)-	Glycidol	Toluene	3-Cyclohexen-1-ol, acetate
Benzaldehyde	Propanoic acid, 2-oxo-, methyl ester	Propanoic acid, 2-oxo-, methyl ester	2,4-Hexadien-1-ol
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	2-Propanone, 1-(acetyloxy)-	2-Propanone, 1-(acetyloxy)-	1,5-Hexadien-3-yne
Phenol	Benzaldehyde	Benzaldehyde	2-Butenal
Cyclopropyl carbinol	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	3-Undecene, (E)-
Isosorbide	Phenol	Phenol	Toluene
Benzofuran, 2,3-dihydro-	p-Cresol	***Isosorbide***	Ethylbenzene
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Cyclopropyl carbinol	Benzofuran, 2,3-dihydro-	p-Xylene
Oxacycloheptadec-8-en-2-one, (8Z)	(*) ***Isosorbide***		Benzaldehyde
	Benzofuran, 2,3-dihydro-		Benzofuran, 2,3-dihydro-
	(*) ***Phytol, acetate***		

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