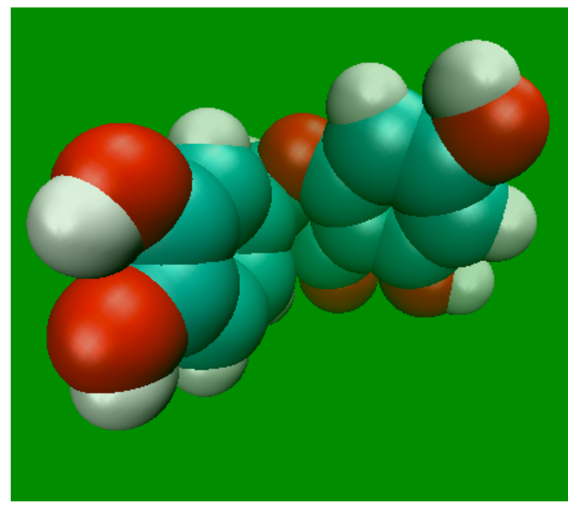
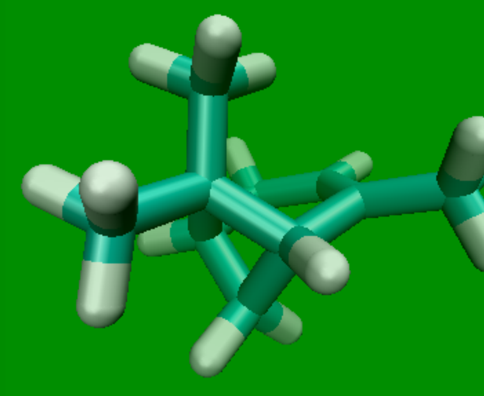


Extractives in Douglas-fir: Tracking Extractives through Biofuel Production and Assessing their Effects on Saccharification



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Abstract

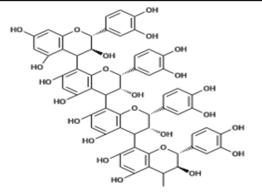
Douglas fir (*Pseudotsuga menziesii*) has been investigated as a feedstock for biofuel processes due to its abundance in western North American timberland. Diverse extractive compounds make up 5% to 25% of the dry weight for different tissues of Douglas fir [1], but are rarely accounted for in biofuel studies. These components are commonly lumped into a lignin, or Klason lignin, category and this category is known to bind to cellulases and obstruct the saccharification step in biofuel production. Extractives may be some of the key culprits of this inhibition. In this work, we identify extractives that are likely to be present in key biofuel process streams and analyze how extractives in the stream to saccharification inhibit cellulases.

Extractives in Biofuel Process Streams and Effluents

Types of Extractives

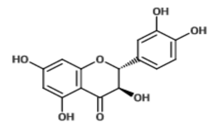
Literature indicates that the most abundant classes of extractives fall into the following groups:

Proanthocyanidin and phlobaphenes



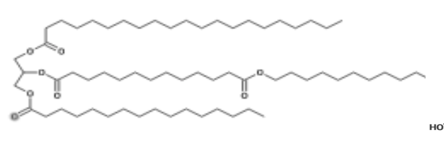
Condensed Tannin

Flavonoids



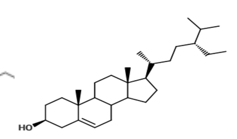
Dihydroquercetin

Waxes



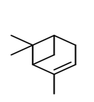
Triglyceride

Phytosterols



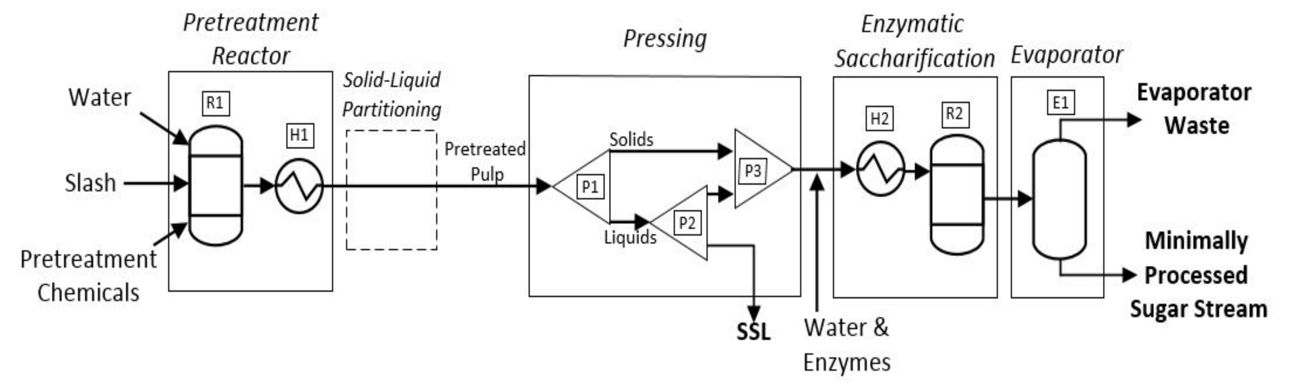
Sitosterol

Terpenes



α-Pinene

Aspen Simulation of Sulfite/Bisulfite Process including Extractives



- An Aspen simulation was assembled that simulates a sugar depot in order to track what streams extractives end up in.
- Common biofuel steps such as Pretreatment, Pressing, Saccharification, and Sugar Concentration (Evaporator) are included.

Extractives in ASPEN Simulation Process Streams

Stream results in kg/hr on a 100kg biomass/hr basis

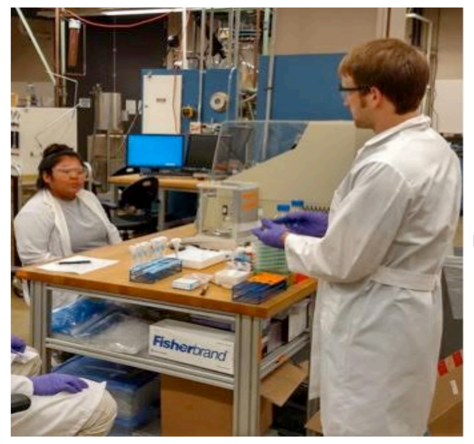
Stream	Feedstock	Spent Sulfite Liquor	Saccharification Feed	Evaporator Waste	Minimally Processed Sugar Stream
Water	400	296.252	423.398	382.16	37.01
Holocellulose (Sugars)	61.919	7.549	52.676	0	56.922
Proanthocyanidins and Phlobaphenes	3.333	2.641	1.126	0	1.126
Flavonoids	2.424	0.81	1.398	0	1.398
Waxes	1.616	0	1.566	0	1.566
Terpenes	0.505	0.079	0.427	.026	0.401
Phytosterols	0.202	0.001	0.18	0	0.18

Methods for Analyzing Cellulase-Extractive Interactions

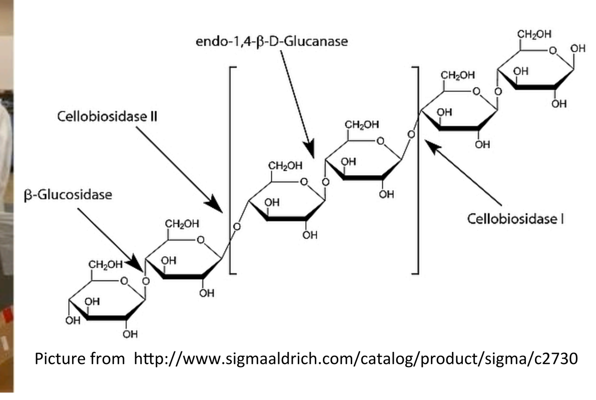
Saccharification Experiments

Saccharification experiments with individual extractives added

- Extractives: Dihydroquercetin and α-Pinene
- Enzymes: Cellulase from Trichoderma Reesei (Sigma Aldrich)
- Substrate: Sigmacell Cellulose
- Saccharification time: 48 hours



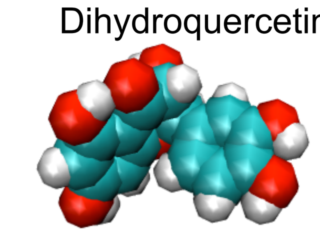
Barbara Wolfin-NARA Summer Intern 2015



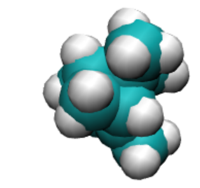
Cellulase-Extractive Binding Simulations

Extractives used in binding analysis

Dihydroquercetin

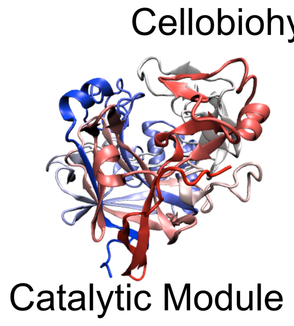


α-Pinene

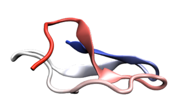


Cellulases from Trichoderma Reesei simulated

Cellobiohydrolase 1

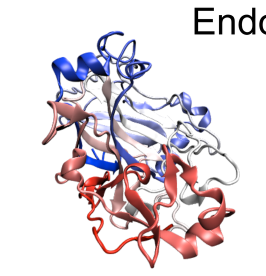


Catalytic Module (1CEL)

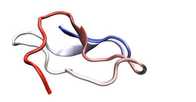


Carbohydrate Binding Module (1CBH)

Endoglucanase 1

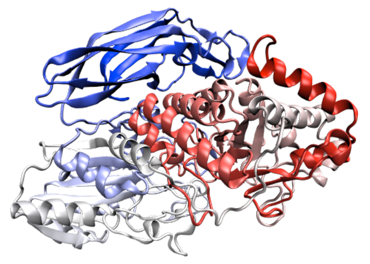


Catalytic Module (1EG1)



Carbohydrate Binding Module (4BMF)

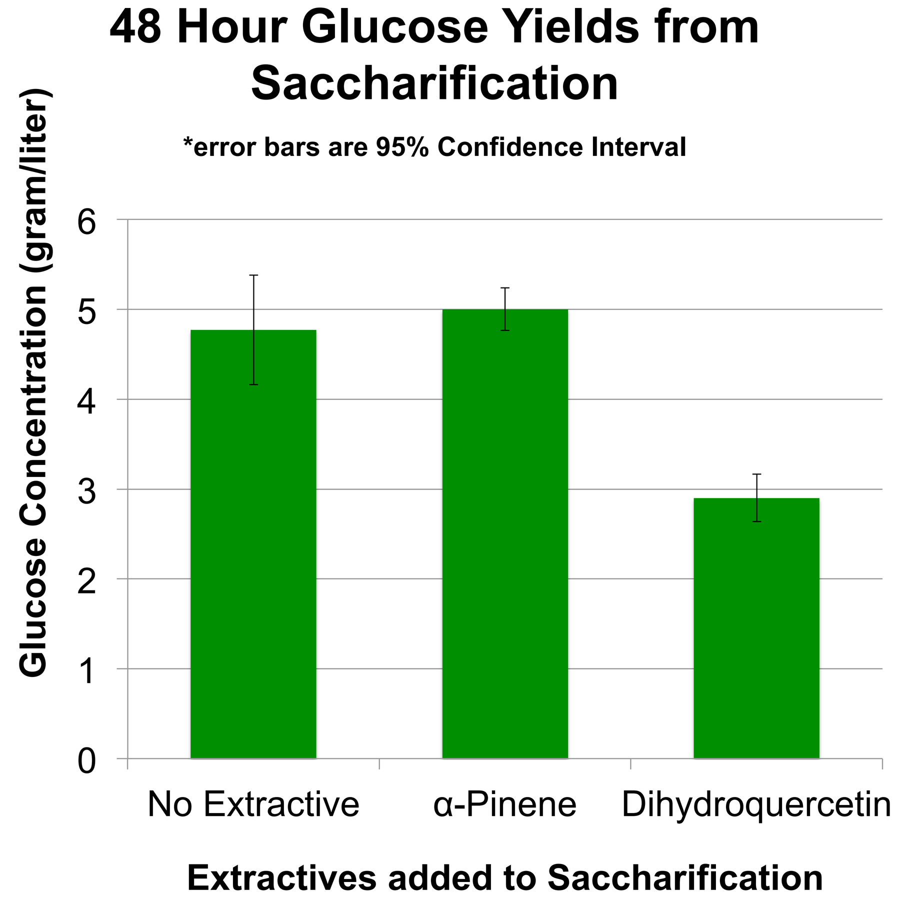
β-D-Glucosidase



(4I8D)

Saccharification Inhibition by Extractives and Extractive-Cellulase Binding

Experimental Results



- Dihydroquercetin inhibits saccharification while α-pinene has no noticeable effect

Binding Simulation Results

Stable site near/at catalytic area

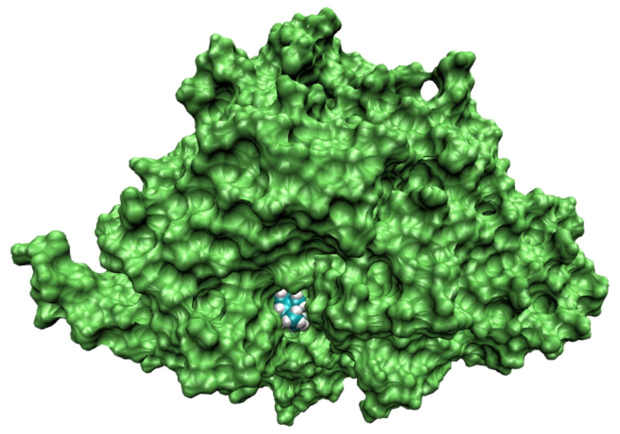
	1CEL	1EG1	4I8D
Dihydroquercetin			
α-pinene			

Stable site on non-catalytic area

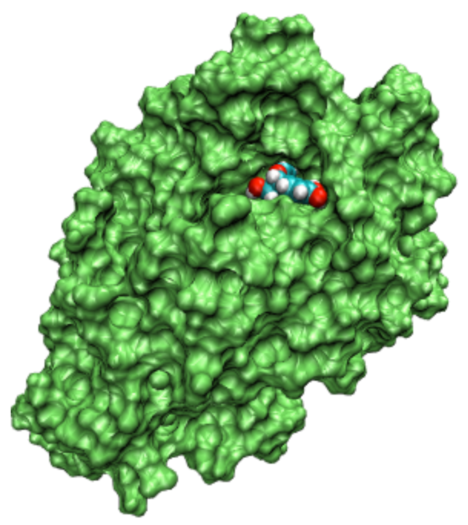
	1CBH	1CEL	4BMF	1EG1	4I8D
Dihydroquercetin					
α-pinene					

Green=at least 1 stable site

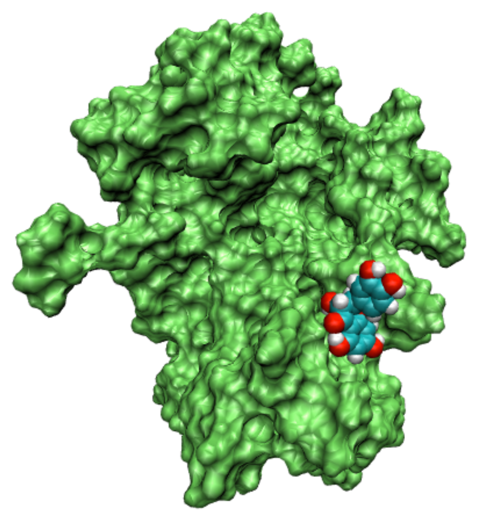
Red= no stable sites found



α-pinene in the catalytic site of 4I8D



Dihydroquercetin binding in the exit of the catalytic tunnel of Cellobiohydrolase I



Dihydroquercetin binding to a non-catalytic section of Endoglucanase I Catalytic Module

Conclusion

- Certain extractives such as dihydroquercetin inhibit saccharification, while other such as α-pinene are relatively inert
- Dihydroquercetin binds to the catalytic module of the three enzymes examined here. On the catalytic module, they can bind to both catalytic areas and non-catalytic parts of the enzyme.
- α-pinene also is stable when placed in the catalytic area for two of the enzymes. However, experimental results imply it is unlikely to travel into the catalytic area in the first place

References & Acknowledgements

References

1. Oleson K, Schwartz D (in Review) Extractives in Douglas-fir Forestry Residue and Considerations for Biofuel Production. Photochem Rev
2. Kumar L, Arantes V, Chandra R, Saddler J (2012) The Lignin Present in Steam Pretreated Softwood Binds Enzymes and Limits Cellulose Accessibility. Bioresource Technol 103 (1):201-208

Acknowledgements

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