Extracting Oils and Fermenting Carbohydrates from the Same Seed Source to Maximize Biofuel Yields

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Abstract

The need for a renewable and clean form of energy to power our transportation is growing as the negative effects of using fossil fuels continues to increase. One solution is the use of biofuels derived from seed sources like corn, primarily used for ethanol, and soybeans, primarily used for biodiesel. Typically, only one type of biofuel would be extracted from a seed, but this experiment attempts and successfully extracts both the oils and ferments alcohol from the same seeds. The experiment not only tested corn and soybeans, but it also tested acorns due to the fact that it is not a food stock. The results are that the acorns provided the highest weight percent of both oil and ethanol with 30.0% extracted out of its flour. The extraction and fermentation yielded a total weight percentage of 27.1% for corn, and 23.0% for soybeans.

Introduction

The demand for alternative energy sources is increasing as the demand for energy and the environmental impacts of fossil fuels have increased. Alternative fuels such as ethanol and biodiesel are viable options to subsidizing the use of fossil fuels due to their cleanliness, renewability, and minimal amount of modifications to current systems necessary to use the fuel. One of the largest crops used for ethanol production is corn due to its high starch and sugar composition. The starches and sugars are converted into ethanol through fermentation. Soybeans are one of the most common crops for biodiesel due to its high oil content. The oils, such as mono-glycerides, di-glycerides, and triglycerides, are converted to biodiesel through a chemical reaction known as transesterification. Using corn and soybeans as an example, they both contain carbohydrates and oils. Corn has oil, but in lower concentration and proportion to soybeans and vice versa. Table 1 shows that in a 100 gram sample, white corn has 67.3 grams of sugars and starches, and 4.7 grams of oils. On the other hand, mature soybeans (100 gram sample) have a sugar and starch content of 20.86 grams, but a much higher oil content of 19.94 grams, ideal for biodiesel production.

Table 1 Data from the U.S. National Nutritional Databases. (w/w)%

	Corn ¹	Soybeans ²	Acorns ³
Fats	4.74%	19.64%	23.86%
Sugars	0.64%	7.33%	no data
Starches	74.26%	30.16%	40.75%

Biofuel production only focuses on extracting only the major component of the seeds, and the rest goes toward livestock feed and other sources that do not create fuel. Soybean oil alone when converted to biodiesel has the potential to reduce greenhouse gas emission by 45% to 75% compared to using only diesel derived from fossil fuels based on a life cycle analysis study. From a similar study, ethanol fermented from wheat has the potential to reduce greenhouse gases, which include carbon, methane, and nitrous oxide, by 52%. There is also the issue of using these seed sources due to the fact that using them can impact the

food stocks in order to meet energy demands. This project combines both oil extraction and ethanol fermentation from the same seed in order to maximize the amount of fuel extracted. As an alternative seed source, acorns were used due to the fact that they are not a human food source, known to have a relatively high oil content, grow without annual planting, and typically they are dumped in compost sites.

Materials and Methods

The seed sources selected for this experiment were organic vellow popcorn from Whole Foods Market in Orange, soybeans from Fleetwood, PA, and acorns from a red oak tree on Cullen Street in West Haven, CT. The first step for each seed was to extract the oils. The whole seeds were turned into a flour using a blender, loaded into a cellulose extraction thimble, and packed until it reach approximately a quarter inch from the top of the thimble (approximately 100g of flour). The acorns had their shells removed in order to eliminate any rotten samples that could be a source of contamination. After weighing, a soxhlet oil extraction apparatus was set up with a heating mantle, a 500ml round bottom flask filled with 300mL of hexanes, a soxhlet extractor loaded with a sock of anhydrous sodium carbonate on top of the thimble of flour, and a water condenser. The hexanes were boiled, condensed above the soxhlet, deposited in the thimble chamber, and the oil hexane mixture reintroduced by flushing back into the round bottom flask. The extraction cycle was run for at least 20 hours.

The apparatus was cooled and the thimble was allowed to sit in a hood until the hexanes were evaporated. The round bottom flask of the oil solution were rotovapped and later placed under high vacuum in order remove all of the hexanes. The oil in the round bottom was then weighed to determine oil concentrations in the seeds.

After the thimble of flour was dry, a 15% (w/v) solution of the extracted oil flour and deionized water was created in a 2L Erlenmeyer flask. The solution was boiled for 10 minutes and stirred vigorously with a magnetic stir bar. A spray bottle of deionized water was sprayed into the flask in order to prevent boil over. The hot solution was then

transferred to a 1L round bottom flask and then allowed to cool to 70°C. While cooling, the pH of the water was tested with litmus paper to check that the pH was in the range of 5.5-6.0 for optimal conditions for the bacterial alpha amylase (BA 100 from Mile Hi Distilling). If the solution was too basic, 1M citric acid was added dropwise. If the solution was too acidic, 1M sodium carbonate was added dropwise. Once the sample was ready, 2.2% (w/w) of the alpha-amylase to oil extracted flour was added to the solution, mixed, and placed in a 70°C water bath for an hour. The mash solution was removed from the bath to cool to 57°C and the pH was then adjusted to about 4.5. At 57°C, 2.2% (w/w) of glucoamylase (GA 100 from Mile Hi Distilling) to flour was added to the solution, mixed, and placed into a 57°C water bath for 1 hour. The mash was allowed to 35°C out of the bath. The nutrient content for the yeast was boosted by adding 0.2mL of a Pasteur salt solution per gram of flour and 0.34% (w/w) of urea to flour.

Once the solution reached 35°C, 0.66% (w/w) of Ethanol Red® yeast (from Phibro Ethanol Performance Group) to oil extracted flour was added to mash and sat at that temperature for 20 minutes. After hydration of the yeast, the flask was sealed with parafilm that had holes to prevent a pressure build up and the mash solution was placed in a 33°C incubator for at least 7 days to ferment. After fermentation, the mash solution was decanted and strained via a 1 gallon paint strainer (Blue Hawk from Lowe's®) into a clean round bottom flask. Boiling stones were added to the filtered mash solution and hooked up to a 300mm vigreux column and still head/water condenser combo. The filtered mash was distilled and 5mL fractions were collected until the still head reached a temperature of 99°C or greater. Each fraction was later analyzed for ethanol content using a Perkin Elmer Clarus 500 GCFID. The internal size standard used was 0.40mL of 2-propanol was used for a 1.00mL of fraction in the vial for analysis. The ethanol to 2-propanol peak areas were compared to a calibration curve that consisted of 1.00%, 4.00%, 10.0%, 40.0%, and 100.0% ethanol. All of the samples tested in procedural and instrumental testing were done in triplicate.

Table 2 Perkin Elmer Claurus 500 GCFID parameters

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Parameters	Settings		
Column	Zobrax ZB-1 phase		
	30M-0.32mm i.d1.0μm		
Injection Volume	0.5μL		
Oven	45°C for 4min isothermal		
Carrier Gas Pressure	He @ 10.0 psi		
Injector Temp	250°C		
Split Control	50:1		
Detector Temp	280°C		
Detector Gases	H ₂ : 45.0 mL/min		
	He: 450.0 mL/min		
Events	@ 0.00min split 50:1		
	@ 1.75min split 1:1		

Results and Discussion

Table 3 Average Oil Extraction data after three trials of each seed

Seed	Flour in Thimble (g)	Oil Extracted	% Oil/Flour
Corn	111.757	3.981	3.563
Soybeans	92.607	17.805	19.221
Acorns	98.531	18.672	18.950

The oil extracted out of all of the seed samples were fairly consistent with the data provided by the U.S. Nutritional Database. Although the database (Table 1) stated that the acorn had the highest oil concentration with 23.86% oil compared to the acorns in our experiment (Table 3) containing only 18.98% oil, this and the other discrepancies in the data comparison can be attributed to the fact that the sample species and growing conditions can vary, causing the yields the be somewhat different. Despite this, the oil data from the database soybeans was less than a 3% error of the experimental data.

Table 4 Average ethanol yield and Combination of oil and ethanol data after three trials of each seed.

Seed	Corn	Soybeans	Acorn
Flour (g)	111.757	92.607	98.531
Oil extracted flour (g)	106.118	75.670	80.551
Average ethanol recovered (mL)	33.4	4.4	13.8
Mass ethanol calculated (g)	26.3	3.5	10.9
Ethanol to Flour % (w/w)	23.6	3.7	11.0
Ethanol & Oil to Flour % (w/w)	27.1	23.0	30.0

The ethanol data (Table 4) showed that corn was the best for ethanol fermentation yielding an average of 23.6% ethanol by weight of flour. Acorns also performed well with an average of 11.0% ethanol. The soybeans did not do so well compared to the other samples with only a weight percent of 3.7%. Based on the database information for soybeans, the ethanol output should have been nearly as good as acorns, but some unknown factor within the beans itself may have contributed to the problem. Despite the acorn only having the second most oil and ethanol, by weight, it had the highest combination of ethanol and oil with 30.0% (w/w). The biodiesel and ethanol standards, corn and soybeans, performed best in their specific fuel specialty, but do not perform as well when combining both types of biofuel.

This experiment could be further optimized to improve the yields of biofuel, especially for ethanol. The oil extraction method with the soxhlet extractor and hexanes is fairly standard, but a different solvent would help improve its environmental impact. During the mash boiling, some of the flour burned on the bottom of the flask and could have affected the ethanol yield. There are several other factors that can be used to improve the yield of ethanol such as using more types of enzymes and improvements in temperature control though out the process.

Conclusion

The experiment was overall a success that it was possible to extract oil and ferment alcohol with the same seed. The seeds in the experiment yielded fuel in their relative concentrations as expected, except for the soybean fermentation. The surprising result was that the acorns performed the best at the combination of ethanol and oil yields. This experiment could greatly improve, especially in the areas in the optimization of the ethanol yields. The process of the combination of oil extraction and ethanol fermentation use the seeds more efficiently, and has the potential to use the energy stored in the seed more efficiently.

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Biography

I am a chemistry and forensic science double major expected to graduate in May 2015. I was drawn into this research because I enjoy the outdoors like biking and running through the forests of the northeast. Being also a gear head (car person), I was immediately drawn to the opportunity to conduct research in biofuel. The use of fossil fuel based resources is neither sustainable nor good for the environment. Making fuel to power our automobiles from sources that we can simply gather in our backyard fascinates me. I have worked on this research since the end of summer 2013 and continue to work on it. I hope after graduation to pursue a PhD in Chemistry.

