Microwave Assisted Extraction of North American Hazelnut

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Abstract

The need for biodiesel that has a low freezing point is especially important in the colder climates found in areas of the Northern United States. Triacylglycerides (TAG's) are the main renewable oil source used to prepare biodiesel that an engine utilizes for energy. A significant problem in using soybean-based biodiesel as an alternative to nonrenewable transportation fuels is that the TAG's of soybean, from which the biodiesel is derived, contain large enough quantities of saturated fats for the biodiesel to cloud at temperatures well above what can be experienced in the winter months of the Northland. As a result, vehicles that utilize soybean biodiesel at these low temperatures can be rendered useless due to plugging of fuel filters. For this reason as well as others, it is important to identify other renewable oil sources which can be used to produce biodiesel with improved cold temperature properties. In order to identify potential new plant-derived oil sources for this purpose, it is necessary to be able to quickly extract oil from small amounts of plant samples. The most widely used method to extract oils from seeds is called Soxhlet Extraction (SE). In this research, it is concluded that Microwave Assisted Extraction (MAE) is a better alternative to SE in that less solvent is used, less seed is required, and less time is needed. TLC and HPLC are used to analyze the components of each extracted oils to ensure the oils that are extracted are ideal.

Introduction

Renewable biofuels help reduce the global dependency on nonrenewable fossil fuels. With global supplies of oil diminishing[1] and demand being predicted to surpass supply,[2] it is essential that renewable fuel sources be exploited to fill this demand. Companies have been utilizing biofuels as a renewable fuel source because it decreases dependence on nonrenewable fossil fuels.[3] With biofuels, a renewable feedstock can be grown in various locations without the need to drill for fossil fuels in sensitive habitats or in the ocean. Though there are many different types of biofuels, the biofuel of interest in this project is biodiesel for use in engines. The main component of biodiesel is the alkyl ester (e.g. methyl ester) of fatty acids. These fatty acid alkyl esters are derived from TAGs, which plants use to store a large amount of potential energy in the form of fatty acids. TAG's consist of an ester backbone with three fatty acid chains. TAG's that are currently isolated from soybean oils are mainly composed of saturated and monounsaturated fats.[3]

The goal of this project was to produce an extraction procedure that is far more efficient than the procedure that is currently being used. The original extraction method was a procedure called Soxhlet Extraction (SE). What SE does in this case is take solid, ground plant seeds, and extracts desirable oils from them by boiling and then condensing solvent. The oil is extracted from the seeds in this extracting procedure. This process is relatively slow as it can take several hours to complete. The procedure being studied is called MAE and has been proven to lessen the time required to extract oils from biological material.[4] How this procedure is performed is significantly less complex and time-consuming than SE. Ground seeds and desired solvent are put into a microwave-safe tube, the solution is then put in the specific microwave, and finally the solution is microwaved at set parameters to extract the oils. As with SE, the solution is then filtered and evaporated to only have extracted oil remaining.[4] The goals of this project were to extract oils at a high yield but using less solvent, less time, an less seeds

while still extracting a majority of TAG. In previous studies, this proves to be true with general plant material but the study does not have results for extracting from seeds for TAGs.[6]

The physical properties of the biodiesel depend in large part on the types of fatty acids found in the TAG from which the biodiesel is derived where, in turn, depends on the plant of the TAG. Biodiesel derived from TAGs obtained from soybeans, for instance, generally has a high percentage of saturated fatty acids such as stearic acid, which causes solidification of the biodiesel at relatively high temperatures. The current source of biodiesel throughout most of the United States, including the Wisconsin area, is soybeans. Soybean-derived biodiesel still tends to gel at approximately 0^{o}C, limiting its use in colder climates.[5] Using soybeans as a feedstock for biodiesel during the winter months is implausible due to that the biodiesel will cloud and will clog the fuel lines of the vehicle. Another problem about using soybeans as feedstock for biodiesel is that it interferes with a food source and use of agricultural land; this causes the prices of soybeans to increase for a consumer who wants to utilize soybeans and for the company that wants to convert them into fuel. For these reasons, among others, it is economically and ecologically beneficial to find a source of TAG to produce biodiesel that does not interfere with an established food source grown on agricultural land and that clouds (begins to solidify) at a low temperature.

Once a suitable source of TAG is identified, the TAG needs to be extracted from the source matrix. Various techniques have been developed for this purpose, most involving some form of solid-liquid extraction, such as SE. But those extractions utilize an open-vessel system which has many disadvantages that MAE, a closed-vessel system, does not. Some of the benefits of MAE that have been studied are as follows: the closed vessel means that the temperature of the solution can be increased due to the higher boiling point of the solution from the higher that atmospheric pressure, less solvent is allowed to escape because the reaction vessel is sealed, because less solvent escapes there is less solvent that is initially required, the reaction is more accurate and more easily replicable, and the reaction time is greatly lessened.[6]

Our previous results have shown that use of SE requires three or more hours to completely extract TAGs from various plant sources. The performance of MAE was evaluated in the current project due to the benefits mentioned above. In the present study, the seed (kernel) of American Hazelnut was chosen as the source for MAE extraction of TAG. The reason behind choosing American Hazelnut is simple – the seeds contain a majority of desirable TAG with trace amounts of other components. Another reason why using these seeds to produce biodiesel is beneficial is that they can grow in non-arable lands. If one wanted to commercially grow seeds for use in biodiesel, the person would not have to plant on arable lands and potentially lower food supply. The person can find an area of land that that is insufficient for growing a food source and plant feedstock for biodiesel without affecting the food supply.

Because MAE causes the time used to extract oil from seeds to be greatly lessened, the time saved could be used elsewhere. The set up time is also very minimal in that the materials used are fewer in number and relatively less complex, thus causing the set up and take down to require less time. There also is no need to cycle through cold water and, due to the test tube being closed, minimal amounts of

solvent will escape the reaction vessel as vapor. In addition to a smaller reaction vessel, smaller portions of seed and solvent are used.

The different solvents that were tested with MAE are Hexane (Hxn), Isopropanol (Iso), and Hxn:Iso mixture. Each of these solvents was tested at varied seed/solvent ratios and held at varied temperatures. These different solvents were tested because, according to studies, Hxn may not be the ideal solvent for MAE.[7][8] Another reason for using these different solvents was because Hxn is transparent to microwaves, so it does not heat up as readily as Isopropanol because of the dielectric constants of the solvents.[6] Hexane has a dielectric constant of 1.89, isopropanol has a dielectric constant of 18.23,[6] and triolein, which is a form of TAG, of ~2.0.[9] A low dielectric constant reveals a compound as non-polar, whereas a high dielectric constant shows a polar substance. In short, microwaves act more readily on polar substances than on non-polar substances.

Thin Layer Chromatography was used with extracted oils and a standard consisting mostly of Triacylglycerides in order to measure the composition of the extracted oils. During the mobile phase, the eluent travels up the thin silicon layer, the stationary phase. While this happens, this causes the components of the oil being tested to separate, depending on the polarity of each component. In this case, the less polar a component is, the further it will travel on a TLC plate, thus causing a greater rf value to be recorded. High-Performance Liquid Chromatography (HPLC) with Evaporative Light Scattering Detector (ELSD) was used to compliment TLC. A Restek Allure silica column was used as the stationary phase. The mobile phase consists of varied gradients of hexanes and acetones. HPLC was allowed to run for 10 minutes to separate compounds. The separation of compounds is dependent on polarity of each specific compound.

Procedure

Microwave Assisted Extraction (MAE).

MAE was carried out with a Discover Labmate (CEM) microwave reactor. Approximately 0.400 g of ground hazelnut seed was weighed into a 25 mL reaction vial. The appropriate volume of the test solvent was then added to the solid, as well as a stir bar, and the vial was sealed and placed in the microwave reactor. The solution was heated to the appropriate temperature and held constantly at that temperature for one minute. After heating, the solution was then cooled until the sample was safe to handle, 50^{o}C. The resulting mixture was then filtered using a 10mL filtering syringe which was filled to the 2mL mark with Celite, and the clear solution was collected in a pre-weighed a 25mL round bottom flask. The residue remaining in the reaction vial was then transferred to a syringe filter for filtration. After the initial filtration was carried out, 3 mL of the extraction solvent was added and the residue was filtered for a second time to ensure minimal oil was left behind. The filtered solution contained in the round bottom was then lowered into a heated water bath, approximately 70^{o}C, and evaporated of solvent with a rotary evaporator until a constant weight was obtained. Depending on solvent, this could take 15-25 minutes. Hexane required less time to evaporate due to a lowered heat of vaporization.

Whereas isopropanol required a longer evaporation time due to a higher heat of vaporization. All tests were done in duplicate.

Thin Layer Chromatography (TLC)

4cm x 8cm TLC plates were used; the stationary phase consisted of silicon backed by plastic. Eluent consisted of 4:1 Hexane:Ethyl Acetate. The plates are compared with a standard of Hazelnut oil which consists mainly of TAGs. The TLC plates were marked with the appropriate analyte and then transferred to a jar that contained the eluent for approximately 10 minutes before being removed. The plates were then transferred to an iodine chamber to further dye the plates. Finally, the separated components were analyzed and compared with the known standard to determine the contents of each analyte.

High-Performance Liquid Chromatography (HPLC)

Purity of TAG was evaluated using an Agilent 1200 series HPLC with Evaporative Light Scattering Detector (ELSD) and a Restek Allure silica column (150 x 3.2 mm i.d., 5 μm) with guard column (12.5 x 4.6 mm i.d., 5 μm) as the stationary phase. Eluent, mobile phase, consisted of a mixture of hexanes (phase A) and acetone (phase B). For each sample, approximately 5μg of analyte was mixed with 0.500ml of heptane. The samples were then transferred to HPLC to undergo analysis. TAG, DAG, and more polar components were separated using a gradient of 96:4 (A:B) held for 1.5 minutes, then to 90:10 in 0.1 minutes and held for 1.9 minutes, then to 50:50 in 1.5 minutes and held for 3.5 minutes, and finally the gradient was returned to 96:4 in 1 minute and held for one minute. Retention times were verified against known samples of soybean oil, hazelnut oil, triolein, diolien, and monoolien.

Results

Extraction Yields

The yield of oil from American Hazelnut by microwave extraction using hexanes, isopropanol, and a 1:1 mixture of hexanes and isopropanol at various volumes and temperatures was examined. The results show differences in extraction yield that are related to these parameters. Results shown below are averages taken from tables A9, A10, and A11.

Effect of solvent.

	Hxn	Hxn:Iso	Iso
% extracted	53.9	64.8	64.4

All results are with a 10:1 ratio at 130°C for 60 seconds. Values taken are %oil extracted through MAE.
Table 1

As shown in Table 1, oil that was extracted through MAE showed a significant amount of oil being extracted. The table also shows that the Hxn:Iso mixture and Iso extract similar amounts of oil. The duplicates in tables A10 and A11 show that there is very little variation, that the largest difference

for 10:1 ratio at 130°C are >1%. One notable difference is that the time each solvent takes to reach the target temperature of 130°C varies significantly. Hxn takes 5-10 minutes, Hxn:Iso mixture 10-15 minutes, and Iso 15-20 minutes. Because the Hxn:Iso mixture can take up to 10 minutes less time than the Iso mixture while still extracting a similar amount of oils, Hxn:Iso mixture is the ideal tested solvent. Though the extracted oil may be relatively high, if the purity of the oil would show a low amount of TAG, the low reading would render MAE inadequate. This is why HPLC is implemented and the purity of the oil is analyzed later.

Effect of temperature.

	Hxn	Hxn:Iso	Iso
70°C	48.2%	63.1%	60.1%
100°C	52.5%	63.1%	59.6%
130°C	53.9%	64.8%	64.4%

All results are with a 10:1 ratio for 60 seconds. Values taken are %oil extracted through MAE.
Table 2

As seen in Table 2, there is a general trend of increased extraction of oils when the temperature used in MAE was increased. When looking at the table, it is clear Hxn is not the ideal solvent to use. Instead, a Hxn:Iso mixture is clearly the best solvent to use in MAE with any temperature. As with the effect of solvent, the duplicates in tables A10 and A11 show that there is very little variation, that the largest difference for 10:1 ratio at 130°C are >1%.

Effect of extraction solvent volume.

	Hxn	Hxn:Iso	Iso
(2:1)	50.3%	60.7%	59.9%
(5:1)	53.3%	63.7%	61.4%
(10:1)	53.9%	64.8%	64.4%

All results are at 130°C for 60 seconds. Values taken are %oil extracted through MAE.
Table 3

When looking at Table 3, there is a clear trend of a greater extraction of oils through MAE when the solvent:seed ratio is increased. Similar to the effect of temperature, a Hxn:Iso mixture is also the best solvent to use in MAE with any ratio provided.

Oil Purity

	Hxn	Hxn:Iso	Iso	Soxhlet

TAG	99.70%	99.75%	99.75%	97.72%
DAG	0.1407%	0.02220%	0.08442%	1.923%
MAG	0%	0%	0.01288%	0%
Unk	0.16%	0.2290%	0.1506%	0.3536%

All results are with a 10:1 ratio and at 130°C for 60 seconds. Values are taken through HPLC.
Table 4

As seen in Table 4, the amount of TAG in oils that were extracted through MAE is significantly greater than the oils extracted through SE. The amount of TAG extracted through SE was 97.72% where the minimum amount of TAG extracted through MAE was 99.70%. The least amount of DAG was found in the Hxn:Iso mixture. The only solvent that showed confirmation for MAG in solution was the solvent Iso. Because of the small amount of MAG in solution and the larger DAG in the Iso solution, Hxn:Iso mixture is the solvent that is ideal to extract the most pure TAG.

	Hxn	Hxn:Iso	Iso	SE
TAG %	53.74	64.64	64.24	64.01

Results are the product of amount of oil extracted though MAE and HPLC TAG results.
Table 5

The solvent: seed ratios indicated in the data presented were calculated by dividing the volume amount of the solvent by the total weight amount of the solution. The temperatures tested were at 70°C, 100°C, and 130°C which are included in the 3D graph. The final values are the percent yields. These values were obtained by taking the weight value of the extracted oils after removing solvent and dividing them by the weight value of the total hazelnut seed that was placed in the extraction vessel before extraction. These values were then entered into Microsoft Excel and rendered in a 3D graph for display of the effect of each parameter. Each solvent showed the same general trend of a low initial reading at 70°C and 2:1 ratio with a significantly higher reading at 130°C and 10:1 ratio. At 130°C with a ratio of 10:1 using Hxn:Iso as a solvent displayed the highest average yield, which is 64.82%.

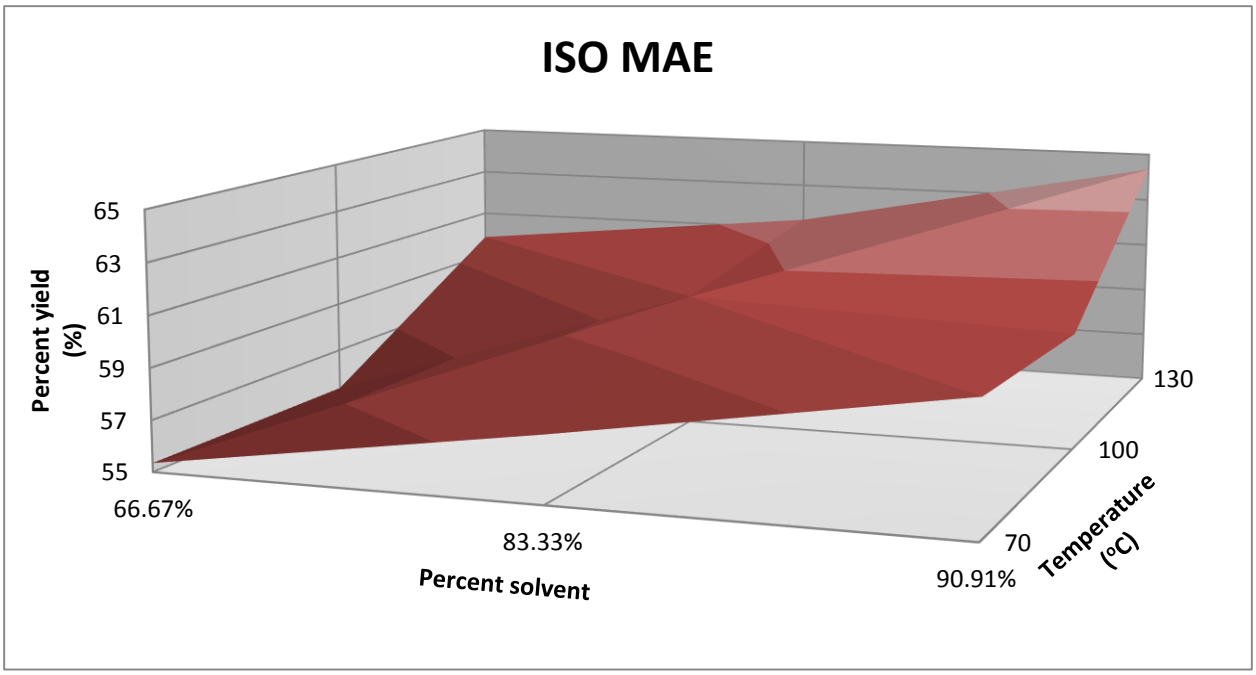


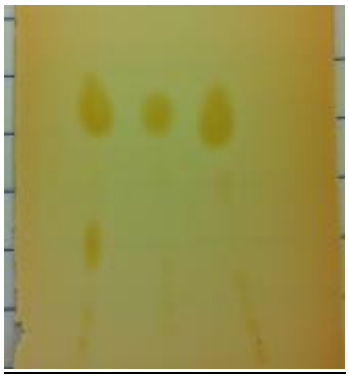
Figure 1

	Temperature (°C)			
Solvent:Seed ratio (v:m)		2:1	5:1	10:1
	70	55.35	57.6	60.05
	100	55.35	60.2	59.6
	130	59.85	61.4	64.35

Table 6

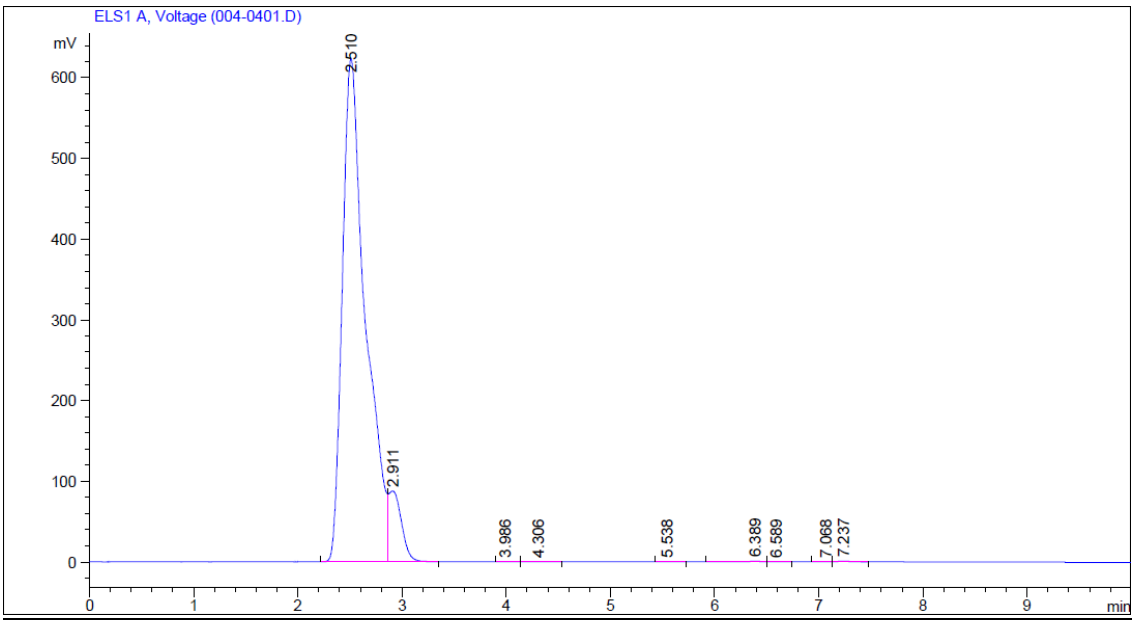
Figure 1 and Table 6 are condensed versions of the above information. Solvent:seed ratios are compared to temperature (°C) and %oil extracted with MAE. The graph shows the same trend through each of the different solvents, a maximum at a 10:1 ratio and a temperature of 130°C with a minimum at a 2:1 ratio and temperature of 70°C.

In addition to measuring the amount of oil extracted under a given set of conditions, it is also important to determine how much of the oil is composed of TAG. This is important because it is the TAG that is converted to biodiesel; other components, if present, can interfere with this conversion and adversely affect the properties of the resultant fuel. That is why TLC, then HPLC are used to analyze the contents of the oil. Though when using TLC the testing will be more qualitative than quantitative, there will be a better understanding of the compounds that are in the oil.



1 – Hazelnut standard, Rf TAG 0.497
2 – Hxn:Iso 10:1 at 100°C, Rf TAG 0.464
3 – Hxn:Iso 2:1 at 130°C, Rf TAG 0.481
Figure 2

Thin Layer Chromatography was the first qualitative test performed. Due to TLC not being quantitative, the results from TLC can only reveal a general idea of what to expect when HPLC is performed. However, TLC with iodine stain has been found to be a sensitive method to detect other oil components that are present in addition to TAG. Each testing of extracted oils with TLC shows very similar results, a majority of the oil appears to be TAG. The reasoning behind this is that hazelnuts are known to contain mostly TAG and in TLC, there is one significant result that is clearly a majority of the solution. With the hazelnut standard that is extracted through SE, there is a secondary significant result at Rf value 0.234 where oils extracted with MAE do not show as significant of a result at the same value. This shows that MAE may potentially extract a higher total percentage of TAG than SE.



Hxn:Iso at 130°C with 10:1
Figure 3

Peak #	Ret Time (min)	Type	Width (min)	Area (mV*s)	Area (%)	Name
1	1.920		0.0000	0.0000	0.0000	Isopropanol alcohol esters
2	2.510	BV	0.2226	9646.6045	92.5710	?
3	2.911	VB	0.1286	747.9802	7.1778	Triglycerides
4	3.986	BV	0.1183	0.9020	0.0087	?
5	4.306	VB	0.1422	3.6127	0.0347	?
6	5.538	BB	0.1008	1.5937	0.0153	?
7	6.389	BV	0.1908	9.1087	0.0874	?
8	6.589	VB	0.0994	2.3174	0.0222	Diolein
9	7.068	BV	0.0887	1.2442	0.0119	?
10	7.237	VB	0.1052	7.3983	0.0710	?
11	8.360		0.0000	0.0000	0.0000	Monoolein

Table 7

When looking at a High-Performance Liquid Chromatograph, one can accurately differentiate compounds due to the lack of overlap between TAG, DAG, and MAG. Due to each TAG not being completely the same, each TAG can have different properties and can show different values with HPLC as can be seen by looking at the triolein graph and the suspected TAG value. When looking at only the HPLC values and the before-mentioned criteria, the TAG amount extracted through MAE was fairly independent of the solvent used in the process. The values of TAG extracted were: Hxn 99.7%, Hxn:Iso 99.75%, and Iso 99.75%*. Compare this with the hazelnut oil extracted though SE, whose TAG extracted is 97.72%, and the outcome is clear that MAE extracts a slightly higher amount of TAG than SE. This echoes soundly with the above results from TLC.

Conclusion

In this study, the ideal conditions for the extraction to take place are: 10:1 ratio, 130°C, and with the use of an Hxn:Iso mixture. Pure Hxn did not extract as much TAG as the other solvents. Hxn is nonpolar where TAG is more polar, which causes the TAG not to readily dissolve into the nonpolar Hxn. Hxn polarity ties into the dielectric constant of Hxn and the use of Hxn when using a microwave. Hexane has a dielectric constant of 1.89, isopropanol 18.23, and triolein ~2.0. Because of Hxn's dielectric constant, Hxn is transparent to the microwaves and is not as affected by microwaves as is Iso, which has a higher dielectric constant. The process in which the microwaves function is that the polar molecules inside the cells of the seed receive the energy from the microwaves and cause the cell to rupture, releasing the desired oils into the solvent. Because Hxn is transparent to microwaves, the heating up for Hxn in the microwave takes significantly longer than when using Iso as a solvent, which is not transparent to microwaves.

Based on TLC and HPLC results, I conclude that MAE is a more than suitable replacement for SE. MAE, which requires minutes to extract and filter, takes significantly less time than SE, which takes many hours. HPLC results show that when MAE is compared to oil extracted from SE, MAE extracts a

higher percentage of TAG and a lower percentage of other compounds that are non-ideal. By extension, MAE shows that it extracts more total TAG than SE. TLC also helped compliment this finding. Table 1 shows that a 1:1 Hxn:Iso mixture is the ideal solvent to use when compared with Hxn and Iso solvents. Table 2 shows which temperature is ideal, which is revealed to be 130^{o}C. Elevating the temperatures tested could further yield even a different result. Table 3 shows a 10:1 solvent:seed ratio is the ratio that extracts the most oils. Table 4 shows that Hxn:Iso has a high TAG purification when extracted, but with no MAG detected and minimal DAG present. Table 5 also shows that the Hxn:Iso mixture extracts the highest amount of TAG while extracting the least amount of impurities.

References

1. Hirsch, Robert L., Bezdek, Roger, Wendling, Robert.; *Peaking Of World Oil Production: Impacts, Mitigation, & Risk Management* (PDF). Science Applications International Corporation/U.S.Department of Energy, National Energy Technology Laboratory. 2005.
2. Nick A. Owen, Oliver R. Inderwildi, David A. King.; *The status of conventional world oil reserves—Hype or cause for concern?* Energy Policy. 2010. 38, 4743-4749.
3. Pradhan, A., Shrestha, D.S., McAloon, A., Yee, W., Haas, M., Duffield, J.A., Shapouri, H.; *Energy Life-Cycle Assessment of Soybean Biodiesel.* Agriculture Economic Report. 2009. 845. http://www.usda.gov/oce/reports/energy/ELCAofSoybeanBiodiesel91409.pdf
4. Mahesar, S. A.; et al. *Application of microwave heating for the fast extraction of fat content from the poultry feeds.* Talanta. 2008. 75, 1240-1244.
5. Krishna, C. R., Thomassen, K., Brown, C., Butcher, T. A., Anjom, M., Mahajan, D.; *Cold Flow Behavior of Biodiesels Derived from Biomass Sources.* Ind. Eng. Chem. 2007. 8846 – 8851.
6. Mandal, V.; Mohan, Y.; Hemalatha, S. *Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research.* Pharm. Reviews. (2007) 1, 7-18.
7. Tir, R.; Dutta, P.; Badjad, A. *Effect of the Extraction Solvent Polarity on the Sesame Seeds Oil Composition.* EJLST. (2012) 114 (12) 1427-1438.
8. Zhang, F.; Rhee, K.; Koseoglu, S. *Isopropyl Alcohol Extraction of Cottonseed Collets: Efficiency and Performance.* Journal of Food Lipids. (2002) 9 147-160.
9. Junghans, A.; Champagne, C.; Cayot, P.; Loupiac, C.; Köper, I. *Probing Protein – Membrane Interactions Using Solid Supported Membranes.* Langmuir. (2011) 27 (6) 2709-2716.

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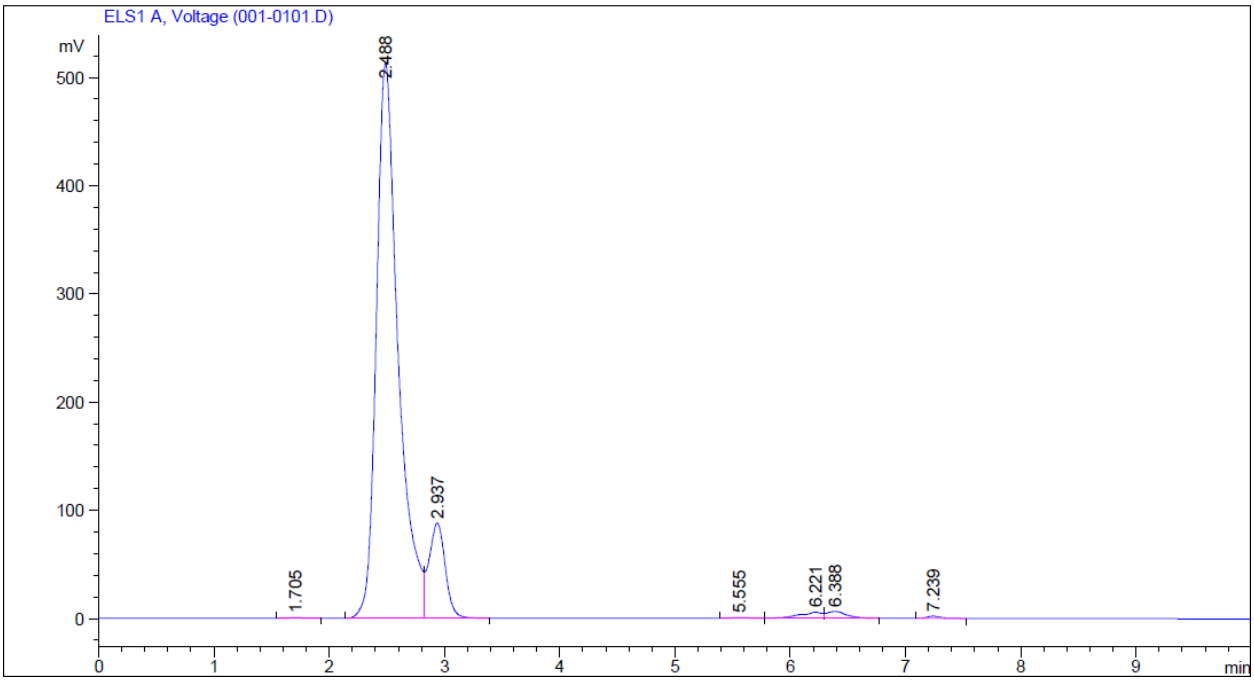
Appendix

HPLC
Hazelnut Oil Standard

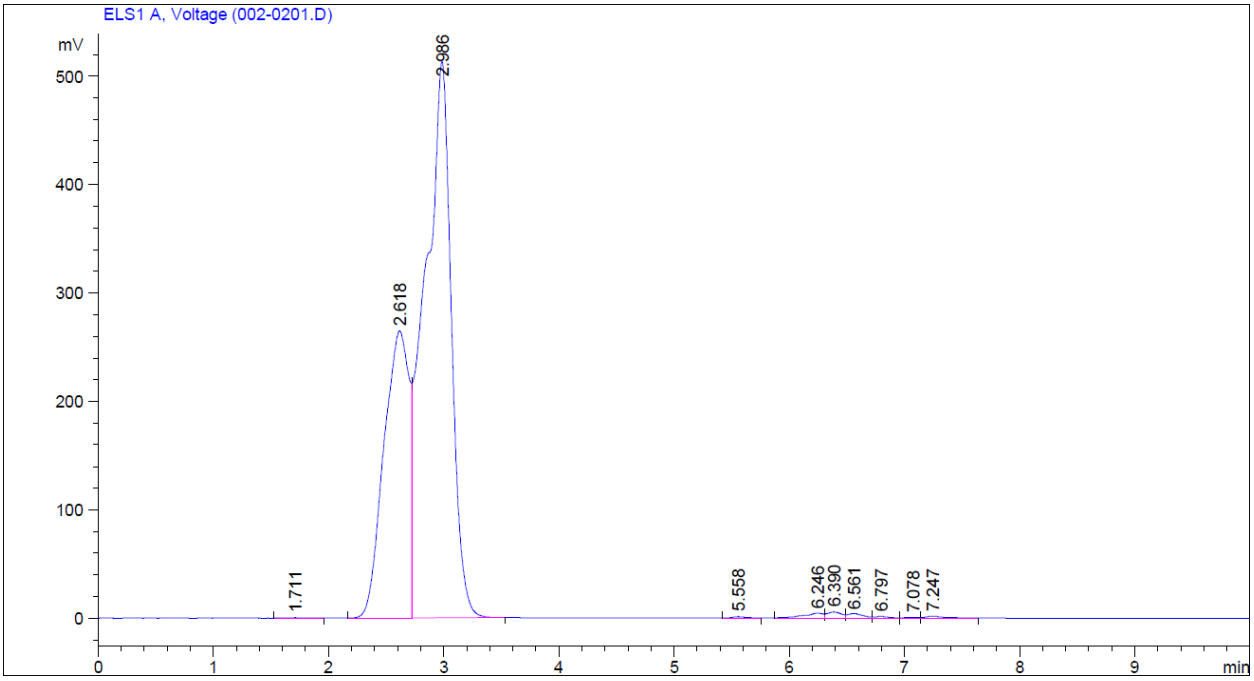


Graph A1

#	Ret Time (min)	Type	Width (min)	Area (mV*s)	Area %	Name
1	1.705	BB	0.1312	5.65038	0.0724	?
2	1.920		0.0000	0.00000	0.0000	Isopropyl alcohol esters
3	2.488	BV	0.1939	6760.99268	86.6634	?
4	2.937	VB	0.1482	862.82397	11.0598	TAG
5	5.555	BV	0.1529	6.05920	0.0777	?
6	6.221	VV	0.1789	74.45720	0.9544	?
7	6.388	VB	0.1751	75.58077	0.9688	Diolein
8	7.239	BB	0.1229	15.87742	0.2035	?
9	8.360		0.0000	0.00000	0.0000	Monolein

Table A1

Soybean Oil Standard

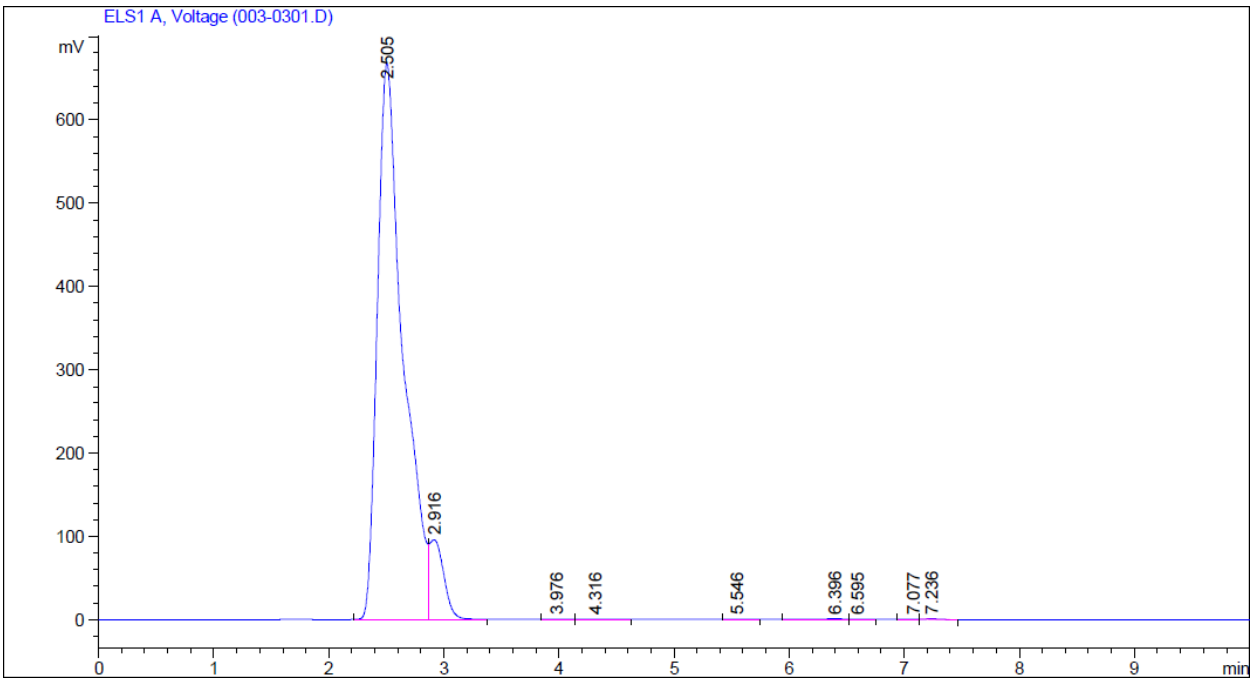


Graph A2

#	Ret Time (min)	Type	Width (min)	Area (mV*s)	Area %	Name
1	1.711	BB	0.1205	4.80501	0.0384	?
2	1.920		0.0000	0.00000	0.0000	Isopropyl alcohol esters
3	2.618	BV	0.2124	4002.16357	32.0155	?
4	2.986	VB	0.2220	8305.87207	66.4433	TAG
5	5.558	BB	0.1226	10.55158	0.0844	?
6	6.246	BV	0.1621	52.69598	0.4215	?
7	6.390	VV	0.1264	48.63083	0.3890	?
8	6.561	VV	0.1381	37.90157	0.3032	Diolein
9	6.797	VV	0.1323	12.27691	0.0982	?
10	7.079	VV	0.1179	6.12925	0.0490	?
11	7.247	VB	0.1617	19.66902	0.1573	?
12	8.360		0.0000	0.00000	0.0000	Monolein

Table A2

Hxn 130°C 10:1

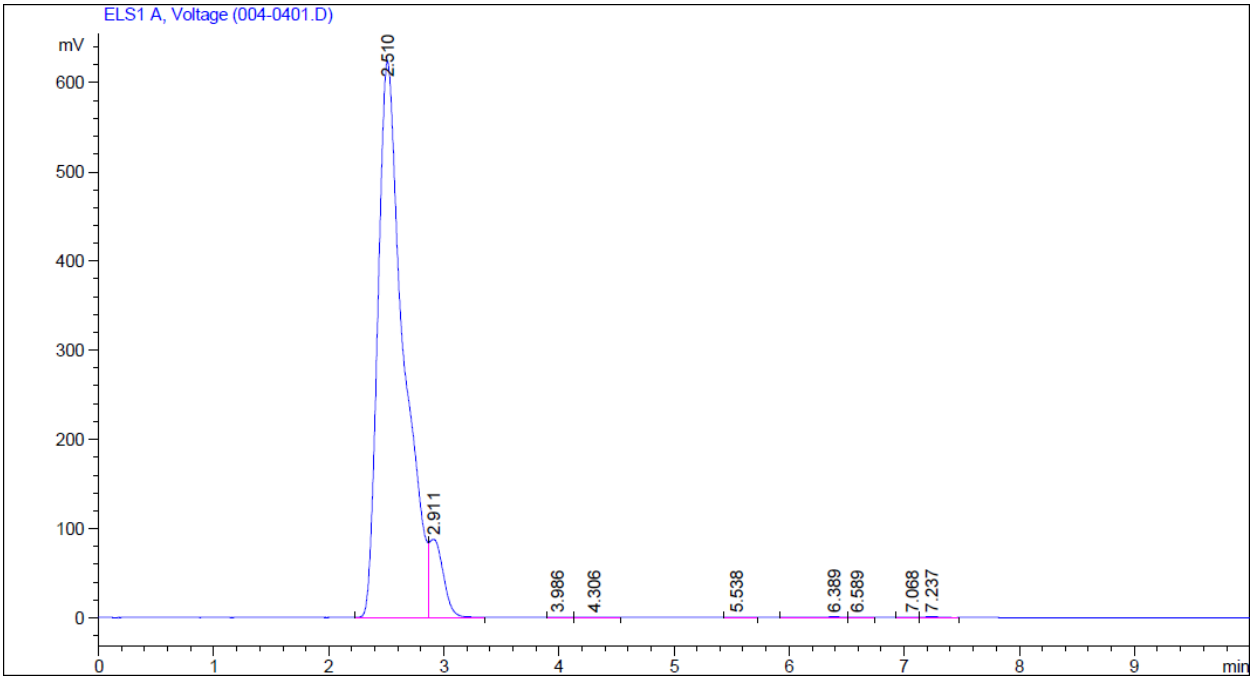


Graph A3

#	Ret Time (min)	Type	Width (min)	Area (mV*s)	Area %	Name
1	1.92		0	0	0	Isopropyl alcohol esters
2	2.505	BV	0.2243	10368.1	92.2105	?
3	2.916	VB	0.135	842.1507	7.4898	Triglycerides
4	3.976	BV	0.1223	1.72862	0.0154	?
5	4.316	VB	0.1722	5.72897	0.051	?
6	5.546	BB	0.1046	1.86071	0.0165	?
7	6.396	BV	0.1764	12.36386	0.11	?
8	6.595	VB	0.1131	3.45816	0.0308	Diolein
9	7.077	BV	0.0842	1.65597	0.0147	?
10	7.236	VB	0.1049	6.89761	0.0613	?
11	8.36		0	0	0	Monoolein

Table A3

Hxn:Iso 130°C 10:1

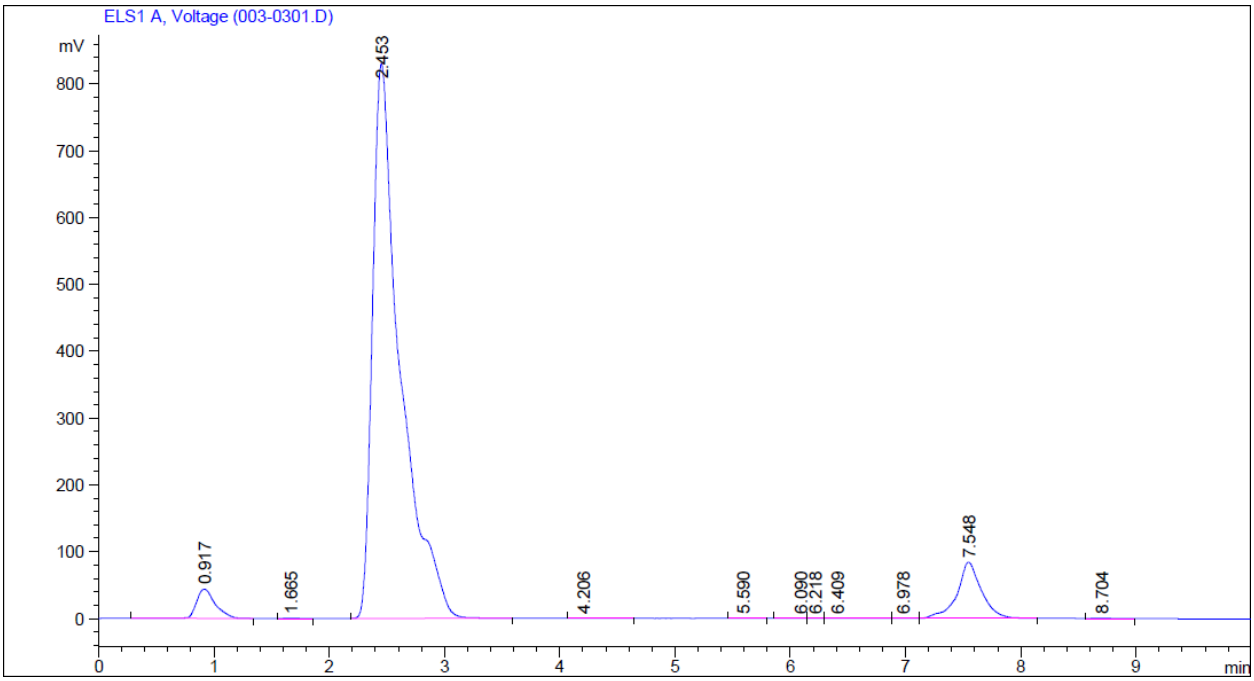


Graph A4

#	Ret Time (min)	Type	Width (min)	Area (mV*s)	Area %	Name
1	1.92		0	0	0	Isopropyl alcohol esters
2	2.51	BV	0.2226	9646.60449	92.571	?
3	2.911	VB	0.1286	747.98022	7.1778	Triglycerides
4	3.986	BV	0.1183	0.901984	0.008656	?
5	4.306	VB	0.1422	3.61266	0.0347	?
6	5.538	BB	0.1008	1.59373	0.0153	?
7	6.389	BV	0.1908	9.10869	0.0874	?
8	6.589	VB	0.0994	2.31736	0.0222	Diolein
9	7.068	BV	0.0887	1.24423	0.0119	?
10	7.237	VB	0.1052	7.3983	0.071	?
11	8.36		0	0	0	Monoolein

Table A4

Iso 130°C 10:1



Graph A5

#	Ret Time (min)	Type	Width (min)	Area (mV*s)	Area %	Name
1*	0.917	BB	0.1675	499.04105	3.2003	?
2	1.665	BB	0.1206	1.27603	0.008183	?
3	1.92		0	0	0	Isopropyl alcohol esters
4	2.453	BB	0.2364	13844.3	88.7812	?
5	2.92		0	0	0	Triglycerides
6	4.206	BB	0.2151	7.00069	0.0449	?
7	5.59	BB	0.1424	2.63986	0.0169	?
8	6.09	BV	0.1545	4.88322	0.0313	?
9	6.218	VV	0.1148	3.67032	0.0235	?
10	6.409	VB	0.2491	11.71593	0.0751	Diolein
11	6.978	BV	0.1064	1.37262	0.008802	?
12*	7.548	VB	0.2004	1216.03687	7.7982	?
13	8.704	BB	0.1127	1.78812	0.0115	Monoolein

Table A5

*#1 and #12 omitted in final calculations

Thin-Layer Chromatography

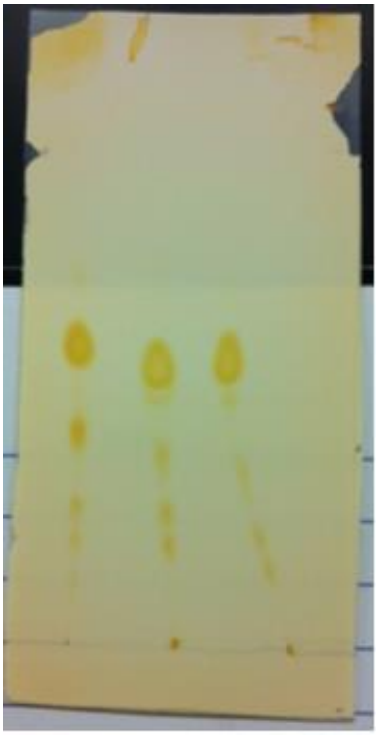


Figure A1
1 – Hazelnut Standard
2 – 2:1 Hx-Iso 100*C
3 – 5:1 Hx-Iso 100*C

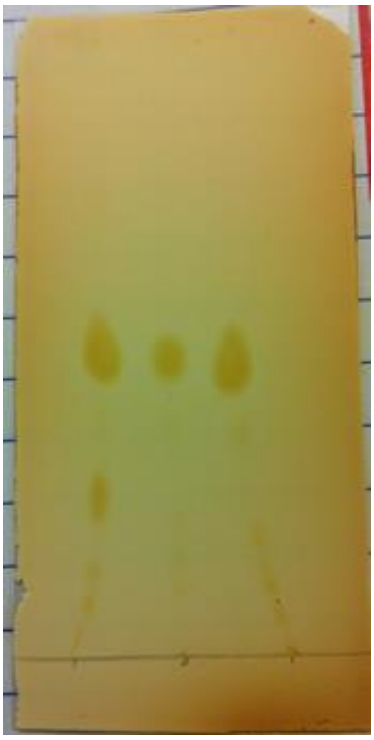


Figure A2
1 – Hazelnut Standard
2 – 5:1 Iso 70*C
3 – 10:1 Iso 70 *C

Figure A3
1 – Hazelnut Standard
2 – 5:1 Iso 100*C
3 – 10:1 Iso 100*C

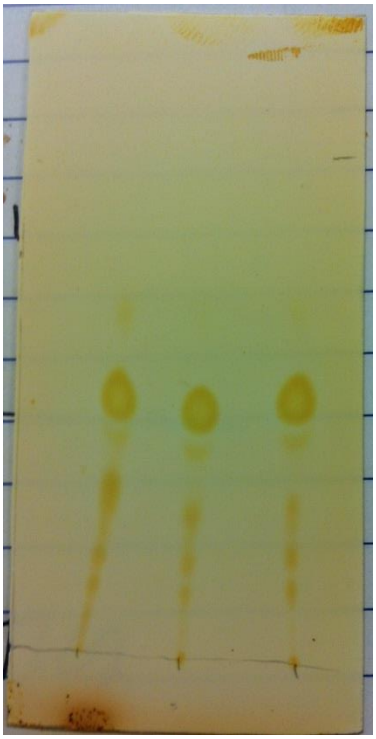
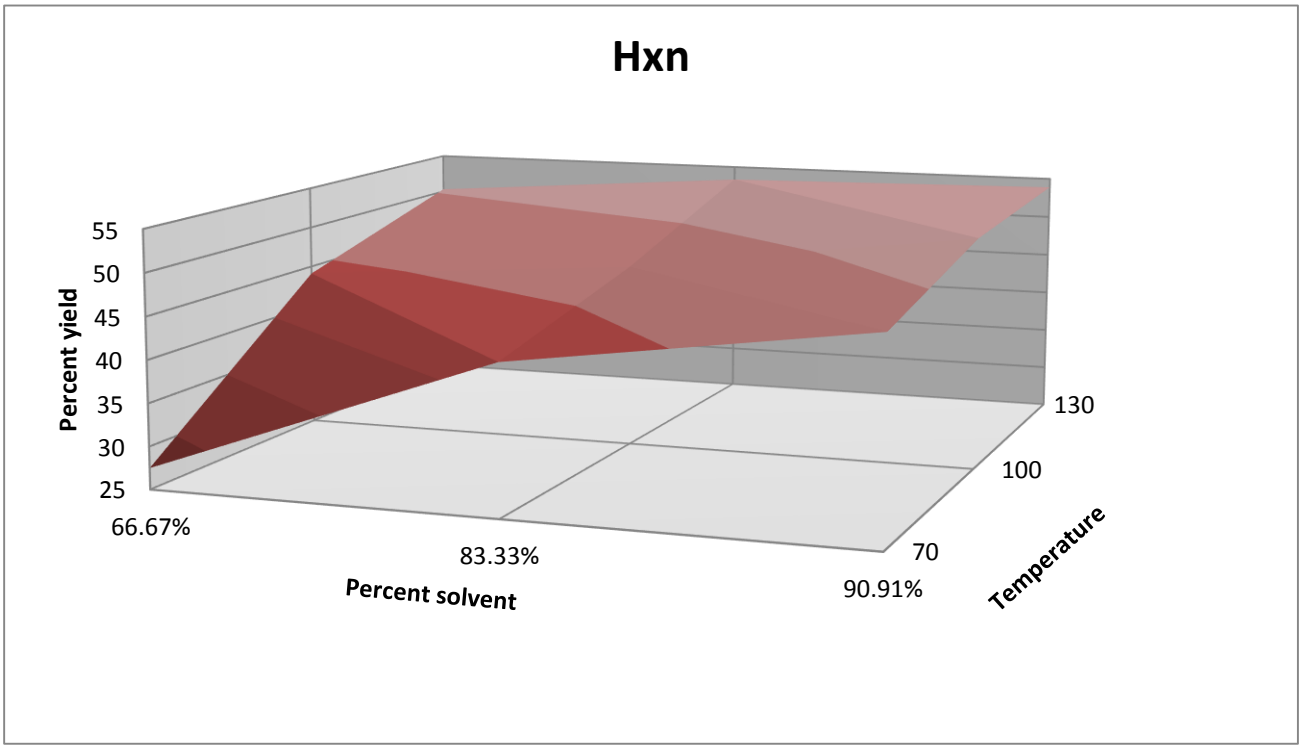


Figure A4
1 – Hazelnut Standard
2 – 5:1 Iso 130*C
3 – 10:1 Iso 130*C

MAE

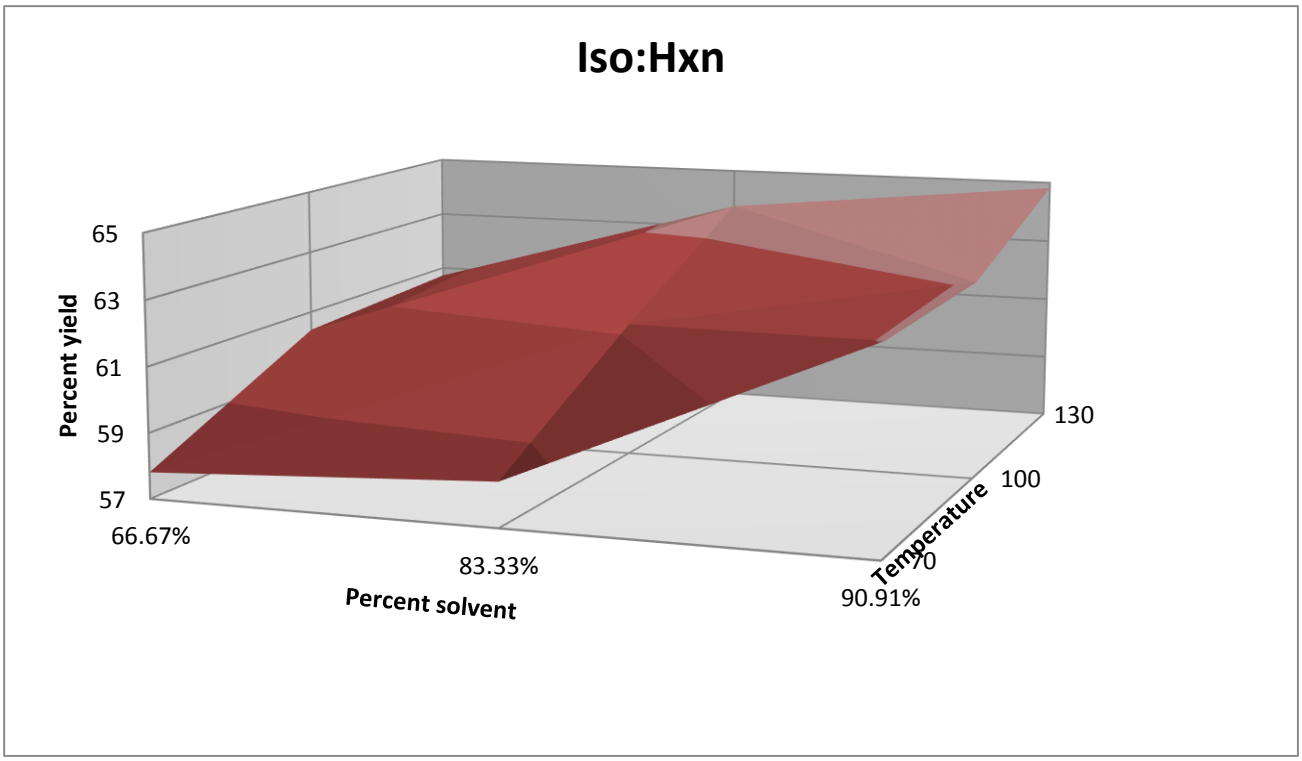
% yield given by oil weight to seed weight



Graph A6

	Temperature (°C)			
Solvent:Seed Ratio		66.67%	83.33%	90.91%
	70	27.5	42.4	48.15
	100	44.1	47.05	52.5
	130	50.3	53.3	53.9

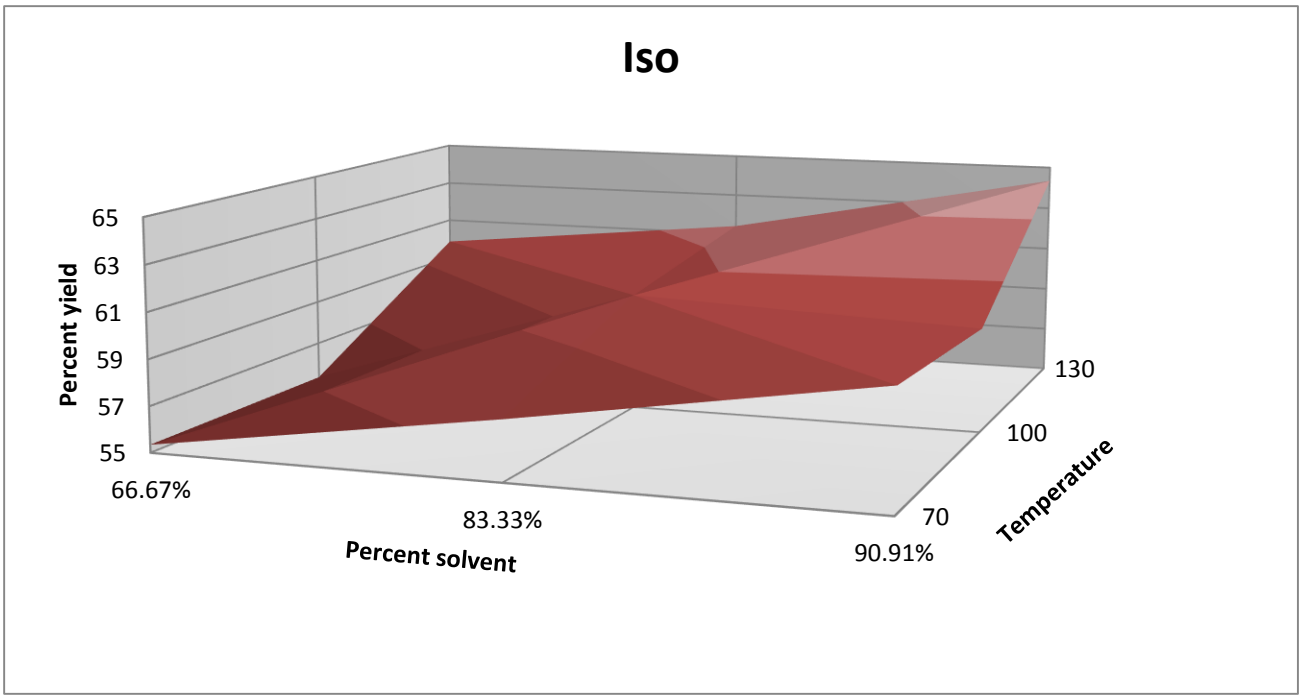
Table A6



Graph A7

	Temperature (°C)			
Solven:Seed Ratio		66.67%	83.33%	90.91%
	70	57.81	58.36	63.06
	100	60.4	61.2	63.12
	130	60.72	63.74	64.82

Table A7



Graph A8

	Temperature (°C)			
Solvent:Seed Ratio		66.67%	83.33%	90.91%
	70	55.35	57.6	60.05
	100	55.35	60.2	59.6
	130	59.85	61.4	64.35

Table A8

Solvent	Temperature	Time (s)	Solvent:Seed Ratio (v:m)	Oil yield 1	Oil Yield 2
Hexane	70	60	(2:1)	28.25%	26.75%
Hexane	70	60	(5:1)	43.75%	40.97%
Hexane	70	60	(10:1)	49.26%	46.98%
Hexane	100	60	(2:1)	45.54%	42.68%
Hexane	100	60	(5:1)	47.13%	47.01%
Hexane	100	60	(10:1)	52.88%	52.10%
Hexane	130	60	(2:1)	50.99%	49.62%
Hexane	130	60	(5:1)	53.90%	52.66%
Hexane	130	60	(10:1)	55.92%	51.86%

Table A9

Solvent	Temperature	Time (s)	Solvent:Seed Ratio (v:m)	Oil yield 1	Oil Yield 2
Hexane Isopropanol (1:1)	70	60	(2:1)	58.52%	57.11%
Hexane Isopropanol (1:1)	70	60	(5:1)	58.84%	57.88%
Hexane Isopropanol (1:1)	70	60	(10:1)	63.34%	62.78%
Hexane Isopropanol (1:1)	100	60	(2:1)	60.79%	60.00%
Hexane Isopropanol (1:1)	100	60	(5:1)	60.95%	61.46%
Hexane Isopropanol (1:1)	100	60	(10:1)	62.81%	63.43%
Hexane Isopropanol (1:1)	130	60	(2:1)	60.65%	60.79%
Hexane Isopropanol (1:1)	130	60	(5:1)	63.52%	63.95%
Hexane Isopropanol (1:1)	130	60	(10:1)	65.34%	64.31%

Table A10

Solvent	Temperature	Time (s)	Solvent:Seed Ratio (v:m)	Oil yield 1	Oil Yield 2
Isopropanol	70	60	(2:1)	54.68%	55.97%
Isopropanol	70	60	(5:1)	58.23%	57.04%
Isopropanol	70	60	(10:1)	60.20%	59.90%
Isopropanol	100	60	(2:1)	56.39%	54.29%
Isopropanol	100	60	(5:1)	59.90%	60.55%
Isopropanol	100	60	(10:1)	59.20%	60.00%
Isopropanol	130	60	(2:1)	59.75%	60.00%
Isopropanol	130	60	(5:1)	61.71%	61.07%
Isopropanol	130	60	(10:1)	64.04%	64.66%

Table A11