An Assessment of Hazelnut Variety on Oil Content and Composition -A Future Alberta Oil Crop

Presented to: Cindy Rothwell -Client

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Abstract

The quality of fourteen hazelnut varieties is measured to differentiate between the levels of oil content per nut, crude protein in mash, and fatty acids in cold press oil. Filbertone in roasted hazelnut is qualitatively assessed to identify the potential of the hazelnut in the current market. The objective of the analysis is to predict the success of each variety in the market place. Some of the varieties are genetically crossed to produce hybrids.

Cold Press method was used to perform statistical analysis of 14 hazelnut Varieties. Samples 10 and 6 produced the most oil per nut than the rest. Sample 10 produced the most oil per kernel than any other variety. Sample 6 had the smallest percent kernel to nut.

The hazelnut mash was analyzed for crude protein content by Kjeldahl's method. Samples 3 show the highest protein content than any other hazelnut variety.

Gas Chromatography Mass Spectroscopy and Fatty Acid Methyl Ester Profile was performed on the cold pressed hazelnut oil varieties. Hazelnut samples are compared to cold pressed olive oils and evaluated for saturated, monounsaturated, and polyunsaturated fatty acid content. Sample 11 has the lowest saturated fat content of the 14 varieties and is lower than olive oil. Sample 6 contains highest levels of mono-unsaturated fats and is higher than olive oil. Sample 6 also has the lowest poly-unsaturated fat, lower than olive oil. Sample 1 has the highest level of poly-unsaturated fats, higher than olive oil.

Fourier Transform Infrared Spectroscopy was used in the comparison of roasted hazelnut oil to non roasted hazelnuts. The results qualitatively distinguish filbertone content. The results show that Roasted Hazelnuts have larger amounts of filbertone than

unroasted nuts. Only quantitative work will allow differentiation of the varieties. Then the best route for product development of hazelnuts can be better estimated.

Introduction

In the later part of the 1900's, many American Cultivars have attempted at increasing the commercial value of hazelnuts by creating hybrid hazelnut varieties. The method of evaluating the quality of hazelnuts being produced must predict the success of commercial production. Variation in oil content, fatty acid composition, and crude protein in the mash will attempt to distinguish between commercial potential and lower quality genetics[1]. In total, fourteen hazelnut varieties will be examined from a Badgersett hazelnut farm.

The Cold Press method is used to extract cold oils from hazelnuts and measure the oil content of the hazelnut varieties. Researchers have found many benefits to cold pressed hazelnut oil such as lowering cholesterol levels and providing a source for essential nutrients[2]. The cold oils from hazelnuts are compared to the quality of Olive oil. Once the cold oils are extracted from the nut, the remaining mash must be utilized to produce the highest profits.

If the amount of recoverable oil from solvent extraction of the mash is large, lower grade hazelnut oil can be sold in the market place[3]. Oil producers are known to blend cold pressed hazelnut oil with solvent extracted hazelnut oils. After methodology refinements with limited apparatus capability, we concluded that this method would not be of interest in regards to an industrial scale application due to possible degradation of the oil as well as the expense of solvents and power to run the extraction. The solvent extraction method was replaced with the protein determination of mash. The total oil content could further differentiate between the varieties. More research is needed to estimate the profit from solvent extraction.

Hazelnut mash from the cold press is then analyzed for crude protein using Kjeldahl method. This source of protein has been proven to be a meal replacement for chickens and used in many commercial food products[4]. The content of protein helps determine the viability of hazelnuts as a future crop in Alberta as the concentration of protein can be used to derive a value for the nuts as a whole. The Kjeldahl method uses a proportional relationship between protein and nitrogen via a conversion factor (shown in the calculation portion). The Kjeldahl method can be broken down into four major steps; Digestion, distillation, Titration and calculations.

Digestion Step

The purpose of the digestion step is to break the structure and chemical bonds that hold a chemical substance down to simple chemicals and ionic structures. Specifically, proteins and other forms of nitrogen are broken down and converted to ammonia. To accomplish this, 0.85 grams of sample are placed on a digestion tube with 10 ml of concentrated sulfuric acid (H2SO4) and 10 ml of hydrogen peroxide (H2O2). A metallic catalyst (Hg), is then added in the form of a tabulate. The digestion tube is placed into a digestion block where it is heated to the boiling temperature of the mixture. Digestion is completed after 45 minutes at 370ºC to 400ºC.

The Distillation Step

Distillation involves separation of ammonia – nitrogen from the digestate. This is accomplished by raising the pH with sodium hydroxide (NaOH). This changes the ammonium (NH4+) ion to ammonia (NH3). Now it is possible to separate the nitrogen by distilling the ammonia and collecting the distillate in a suitable trapping medium. Collection of ammonia is done by absorption into a solution of four percent boric acid. The ammonia is bound to the boric acid in the form of ammonium borate.

The Titration Step

Determination of the amount of nitrogen on the condensate flask can be accomplished by several methods. The most common is titration of the ammonia with a standard solution of one-tenth normal hydrochloric acid (0.1 H HCl) in the presence of mixed indicator.

The mixed indicators (bromocresol green and methyl red) are available in the four percent boric acid solution.

Calculation

This calculation can either be performed as percent nitrogen or percent protein. For percent nitrogen:

$$\%\ N = \frac{14.01 \times (mL\ titrant - mL\ blank) - (N\ of\ titrant) \times 100}{Sample\ Wt.\ (grams) \times 1000}$$

It has been shown that protein is between 16% and 19% nitrogen. By dividing 100 by 18.75, we get the conversion factor for nitrogen to protein of 5.30. Hence, the percent protein is calculated as follows:

% Protein = 5.30 x %N

The overall chemical reactions involved are

Sample Digestion

Organic N + H_2SO_4 + Heat + Catalyst => CO_2 + H_2O + NH_4HSO_4

Neutralization of Digestion Mixture and Release of Ammonia

NH_4HSO_4 + 2NaOH => NH_3 + Na_2SO_4 + H_2O

Direct Titration of Ammonia

NH_3 + HCl [or H_2SO_4] => NH_4Cl [or $(NH_4)_2SO_4$]

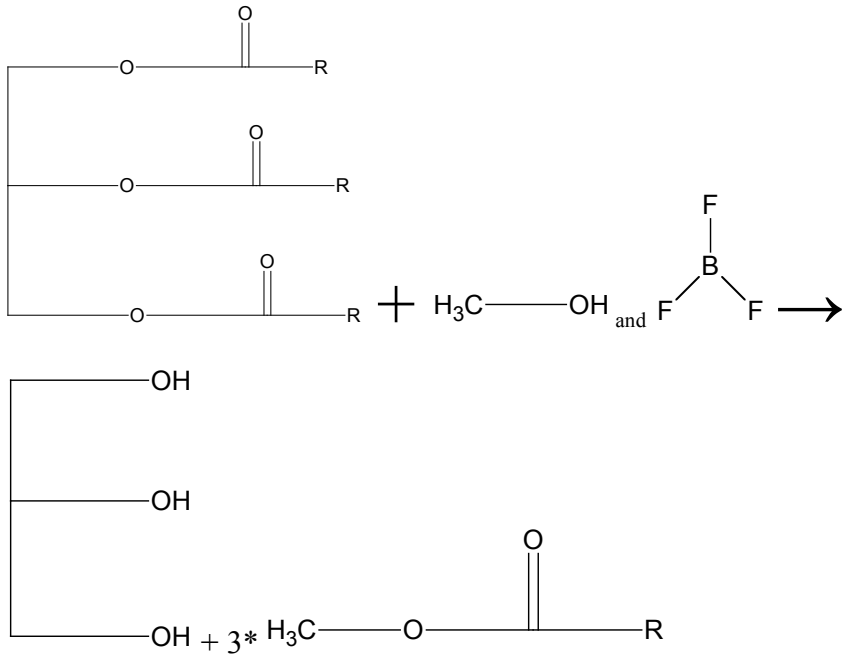
Back Titration of Standardized Acid

NaOH + HCl [or H_2SO_4] => NaCl [or Na_2SO_4] + H_2O

Gas Chromatography Mass Spectroscopy is used to quantity the Fatty Acid Methyl Esters by a two point external standard calibration, using SIM mode. The objective will be to differentiate be the varieties in terms of each standard and comparing them to olive oil. Using the hazelnut oil obtained from the cold press extraction, the triacylglycerols contained within the oils are derivitized.

Esterification is the process reacting carboxylic acids with a catalyst and alcohol to produce an ester. The Esterification process replaces an alcohol group with the –OR group. The result is a fatty acid without the hydrogen bond.

The hazelnut oils are derivatized with boron trifluoride (in methanol), a fast reacting catalyst. The boron trifluoride is added to the hazelnut oil and incubated at high heat, the reaction of this is shown below:



The heat and the catalyst work together to esterify the oils into fatty acid methyl esters. Boron trifluoride (BF_3) is a reactive metal and therefore it is necessary to decompose it in the hazelnut sample. Hexane and water are added to the derivatized sample, inverted and then two layers result. The bottom layer consists of water and the boron trifluoride (in methanol), and the top layer consists of the hexane and fatty acid methyl esters.

The variety of hazelnut samples are now prepared and ready to be injected in the GC-MS. A 1/5 dilution is made of the fatty acid methyl ester hazelnut samples. The varieties of hazelnut oils obtained and esterified have a wide range of concentration of fatty acids, therefore it is necessary to have concentrated and dilute samples to allow for more accurate quantification.

The GC-MS is a gas chromatography instrument with a mass spectroscopy detector. This instrument allows the analyst to separate the ion components of the fatty acid methyl ester hazelnut samples based on their ability to become retained on the stationary phase of the chromatographic column. The MS detector distinguishes between ions based on their m/z (mass to charge) ration. The chromatogram obtained from the GC-MSD is abundance vs. retention time. The abundance is based upon the most abundant peak of the chromatogram, and all other peaks integrated are measured in comparison to the base peak.

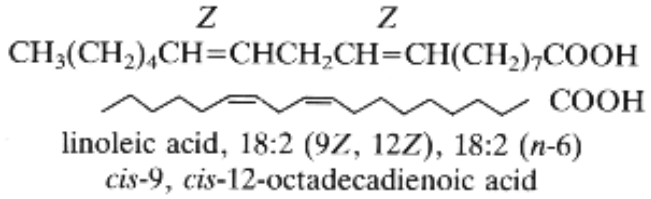
The computer software collects and stores data, and allows manipulation of data. The computer software used for analysis is the SIM mode. SIM mode allows the analyst to decide in advance the specific masses to be monitored. This is essential to monitor the fatty acids present in the hazelnut samples. The target ion can be monitored for a long period of time; this is known as, "dwell time." The dwell time therefore collects a representative number of data points per sample by scanning during the specified time calculated.

The variety of hazelnut samples are being quantified specifically for fatty acids 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 22:0 and 22:1. The following table displays the Carbon Atoms and number of Carbon double bonds, structure and common name of each fatty acid methyl ester.

Table 1: Fatty Acid Methyl Esters Present in Standards

Carbon Atoms:Double bonds	**Structure**	**Common Name**
16:0	$CH_3(CH_2)_{14}COOH$	Palmitic acid
16:1	$CH_3(CH_2)_5C=C(CH_2)_7COOH$	Palmitoleic acid
18:0	$CH_3(CH_2)_{16}COOH$	Stearic acid
18:1	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Oleic acid
18:2	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$	Linoleic acid
18:3	$CH_3CH_2(CH=CHCH_2)_3(CH_2)_6COOH$	Linolenic acid
20:0	$CH_3(CH_2)_{18}COOH$	Arachidic acid
20:1	$CH_3(CH_2)_7CH=CH(CH_2)_9COOH$	Eicosenoic acid
22:0	$CH_3(CH_2)_{20}COOH$	Behenic acid
22:1	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	Erucic acid

Fatty acids are referred to numerically, such as: 18:0, 22:1 or 18:3. The first number represents the number of carbons in the fatty acid chain, and the second number represents how many double bonds the fatty acid has. For example, 18:2, most commonly known as Linoleic acid, is an 18-carbon log chain, with two double bonds.



linoleic acid, 18:2 (9*Z*, 12*Z*), 18:2 (*n*-6)
cis-9, *cis*-12-octadecadienoic acid

courtesy: http://www.cyberlipid.org/fa/acid0001.htm

Essential Fatty acids are fatty acids that the human body cannot synthesize, and therefore must be obtained through the diet. The essential fatty acids are 18:2 and 18:3, because the body is not capable of synthesizing any double bonds.

Long chain fatty acids are commonly between 12- 21 carbons long. Saturated fatty acids contain no double bonds, in specific Hexadecanoic acid (Palmitic acid) is quantified. Saturated fatty acids play a major role in the human body by supplying energy and, are used in hormone production. Most saturated fats are obtained throughout the diet and if they are not, then the body can become out of balance due to lack of growth.

Monounsaturated fats are fats that contain a single double bond. The monounsaturated fats quantified in this experiment are 16:1 (9(Z)-Palmitoleic acid), 18:1 (9(Z)-Oleic acid), 20:1 (11(Z)-Eicosenoic acid) and 22:1 (13(Z)-Docosenoic acid). These fats help reduce heart risk and are obtained in the diet through oils.

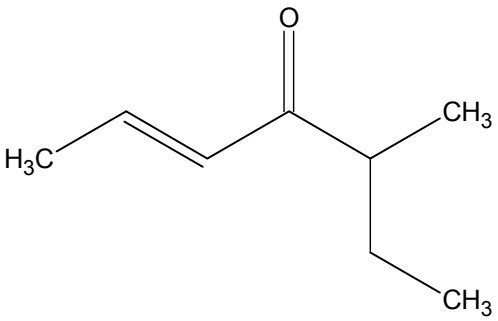
Polyunsaturated fats are fatty acids that contain 2 or 3 double bonds. 18:2 and 18:3, known as Linoleic acid and Linolenic acid respectively. The presence of the essential fatty acids increases the demand and marketable quality of the hazelnut oils.

Mostly essential fatty acids come from seafood, flax and canola, and are present in the oil. There is a high demand for oils with high contents of 18:2 and 18:3 in the food market currently. This experiment will help determine which hazelnut samples, contain high levels of 18:2 and 18:3, enabling producers to sell more hazelnut oil. Supporting the

fatty acid and essential fatty acid content in hazelnut oil, creates a wider market for hazelnuts in the food industry.

Essential fatty acid intake is important for both male and females. A regular balanced diet with the intake of essential fatty acid is helpful to the human body in multiple ways. Essential fatty acids increase the body's energy, increase the immune system, aids in the digestion and increase the healing process. The human body cannot synthesize polyunsaturated fats and therefore they must be obtained through the diet. Therefore the higher % of polyunsaturated fats the better, however, as the % of polyunsaturated fats increase the shelf life decreases. This decrease in shelf life is due to the increase in easily breakable bonds present in the oil which are susceptible to oxidation.

The purpose of using this project is to determine which hazelnut oil variety is the future Alberta oil crop. It is necessary to determine which fatty acids are essential, distinguish between monounsaturated, polyunsaturated fats and saturated fats within each hazelnut sample. This will allow the analyst to place the importance of each hazelnut sample based on their results.



(E)-5-methyl-hept-2-en-4-one (Filbertone)

(E)-5-methyl-hept-2-en-4-one (Filbertone) content is also critical in determination of commercial potential. Filbertone is responsible for the taste quality in hazelnuts. Roasting of the nuts will bring about filbertone more so than in raw nuts. Thus a comparison of roasted hazelnut oil and non roasted hazelnut oil will be accessed to

evaluate the quality of locally purchased hazelnut. Fourier Transform Infrared Spectroscopy will allow the qualitative evaluation of roasting on a specific locally purchased Hazelnut. The flavor of hazelnuts is one of the most important aspects for commercial sales and market assessment[5].

The FTIR method subtracts the spectra of roasted hazelnut oil from non roasted oil. The subtracted spectra should have the conjugated carbon oxygen stretch of ketone around 1660cm^{-1}. If roasting produces greater quantities of filbertone, the subtracted spectra will help influxuate detail which allows accurate qualitative work.

A more accurate method would be to quantity the filbertone by calibrated standard method. The costs involved are great which limited the group to qualitative work. The qualitative method needs significant improvement to control the amount of oil on the salt plates. More research is needed to improve FTIR methods. Then a differentiation of Filbertone could be applied to the varieties. The assessment of filbertone content would allow the comparison of all two routes of product development. Either the entire roasted nut is sold or the oils, mash, and shells created through cold press method.

Experimental

Standard Operating Procedure Cold Press Method Procedure

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Edition 1

Materials: Carver Press Model C serial #24000 553 provided by University of Alberta. 8 inch by 8 inch by 2 inch metal plate provided by Materials Engineering Technology NAIT. Syringe and filter adapter with filter number 1213768. Plastic bags, 1 mL vial and cap, pasteur pipet and bulb, gloves, and nut cracker. Use distilled water, and hexane to rinse.

1. Pre-weigh pasteur pipet, filter #1213768, syringe, and vials
2. Label one plastic bag with trial number and sample number using a permanent marker. Place a new bag inside the labeled bag. Weigh empty bags.
3. Prepare the syringe and filter by, cleaning syringe and needle with soap and water then rinse with reagent grade hexane
4. Accurately weigh each nut using a digital balance.
5. Weigh each kernel obtained after cracking, crack approximately 6 kernels. (Depending on the kernel size may need up to 12). Prevent kernel from dropping on the ground by sealing your hands around the nut while cracking.
6. Place kernels, shells, and remaining nuts on printed sheet for documentation.

7. Take a picture with a digital camera.
8. Place approximately 6 kernels in pre-weighed plastic bags and measure the mass of bags with kernels.
9. Place sample bags on a 8 by 8 inch metal block on the press plate.
10. Rotate knob on the press clockwise until secure.
11. Ensure all fingers and any other obstructions are cleared away between the press.
12. Jack the lever until a pressure of 6 tonnes is reached
13. Release the pressure by rotating the knob counter-clockwise until secure
14. Gently lower the press plate by turning clockwise, to a height where the sample specimen can be retrieved.
15. Remove sample bag.
16. Using a pre-weighed pasteur pipet, extract oil and placed into syringe. May need ruler to maximize extraction.
17. Fill syringe to no greater than the capacity of the vial
18. Re-weigh bags when no more oil can be extracted.
19. Place pre-weighed 0.22 micron filter # 1213768 in adapter and close
20. Attach to syringe
21. Place pre-weighed vial under syringe
22. Filter oil into vial. Is possible to fill vial with all trials to minimize oxidation.
23. Re-weigh sample vial, syringe, filter, and pasteur pipet.
24. Purge vial with nitrogen using an adapter
25. Label a tag with sample name, date, analyst, mass of oil
26. Wrap vial in aluminum foil and attach tag with tape.
27. Store in a cool place
28. Seal and store bags into freezer.
29. Repeat steps 1 through 28 for the remaining trials, and varieties.

Solvent Extraction

The content from the sample bag remaining from the cold press extraction are quantitatively transferred into Dean Stark apparatus. Using 200 mL of hexane and a reflux ratio of 55, the system is refluxed for a minimum of 2 hours and up to 48 hours. Then after cooling system, the solvent is transferred into a 500 mL round bottom flask and the mash is weighed. The round bottom is placed into drying oven at 110 degrees Celsius. The mass is obtained after 15 minute intervals until constant mass is achieved.

1. Weigh accurately mass of nut mash.
2. Accurately transfer mash into pre weighed thimble.
3. Weigh accurately mass of 250ml round bottom flask.
4. Place 100ml of hexane and several anti bumping chips in round bottom flask.
5. Quantitatively transfer nut mash into thimble, assemble Soxhlet apparatus.
6. Reflux for 2 hours.
7. After reflux period rotovap to remove hexane solvent.

Removal of residual hexane and determination of final mass of oil

1. Weigh round bottom flask.
2. Bring round bottom flask and thimble to 70 degrees Celsius for 15 minutes.
3. Cool and reweigh thimble until constant mass is achieved.
4. Cool and reweigh round bottom flask.
5. Repeat until an increase in mass (due to oxidation of oil) has occurred.
6. Take the previous value to the increase in mass for calculations.

Crude Protein

Protein in Hazelnut mash was determined in accordance to Northern Alberta Institute of Technology Course Pack 1619, Food & Agriculture Analysis Laboratory Manual, Pages II-11-1 to II-11-5.

Additions: 5.30 grams protein per gram nitrogen is compared to the stated value of 6.25. Blanks of weigh paper were used as blanks to calculate sample. Ammonium chloride used as a control. 10 mL of 3.05g/mlSodium Thiosulphate Penta Hydrate was added to the boric acid solution before distillation, when a Kjeldahl Tab containing Cobalt was used.

Fatty Acid Methyl Ester Profile

Modifications of NAIT Course pack CHS468 Lab Manual, experiment 14 are discussed.

Experimental Procedure for Derivitization:

1. Make the derivatzing solution by mixing 2.5 mL of boron trifluoride (BF_3 (14% in methanol)), 2.0 mL of toluene and 5.5 mL of methanol together.
2. Weigh about 15 mg (to 4 decimal places) of hazelnut oil into separate 5mL Reacti-vials.
3. Add 1.0 mL of derivatizing solution to each Reacti-vial.
4. Seal the Reacti-vials and place in a metal block heated to 100^{o}C for 30 minutes.
5. Cool Reacti-vials to room temperature.
6. Add 1mL of water and 2 mL of hexane to each Reacti-vial once cooled.
7. Shake the vials and allow the two layers to separate.
8. Collect the upper layer and place in a separate vial for storage.
9. When ready, inject the derivatized sample from the second vial into the GC-MS.

Experimental Procedure for HP GC 5890 MSD:

1. Open ChemStation software, open file FAME 150 program
2. Under Acquisition, fill in all fields (operator name, data file, save as, misc. info)
3. Fill syringe with 1 µL sample + 5 µL air
4. Inject contents into injector port A
5. Press start on the computer screen
6. When the Solvent Delay message appears, Respond: No
7. While chromatogram is running, go to View → Data Analysis → Snapshot
8. When chromatogram is complete (23 minutes runs), integrate accurately by changing peak width, baseline now, baseline back and threshold appropriately

Temperature Program and GC-MSD Information:

Instrument make: Hewlett Packard **Model:** 5890 MSD **Type:** MSD
Gas Cylinder Pressure (psi):
Helium: 65 Hydrogen: 45 Nitrogen: 45 Air: 75

Temperature ^{o}C:
Detector: 280 Injector: 250 Oven: 150

Column:
Length: 25 m Diameter: 0.22 mm Thickness: 0.25 um
Stationary phase: 70% cyanopropylsiloxane

Sample:
Volume: 1 µL sample + 5 µL air Injection method: normal

Filbertone Roasting Comparison

Fourier Transform Infrared Spectroscopy was used to determine difference in Roasting on locally purchased Nuts. Filbertone will be monitored qualitatively by subtracting from olive oil spectra. A weighted solvent subtraction and air background subtraction will be performed for accuracy purposes. The filbertone peaks are then differentiated.

FT-IR Procedure

1. Open FT-IR Nicollet
2. Click view experimental set up
3. Enter 100 scans and 4 cm^{-1}
4. Click into accessories
5. Change to transmittance accessory
6. Save background file to specific location
7. Collect air background
8. Transfer 10 or 20 micro liters of oil onto salt plates delivered by micropipette.
9. Collect sample
10. Change to absorbance
11. Save spectra
12. Clear window and repeat
13. Open TQ Analyst
14. Open Roasted Oil file
15. Open Non roasted file

16. Click edit and select both files
17. Click tools and subtract spectra
18. Determine the best weighting function
19. Adjust spectra for comparing
20. Click add
21. Print Spectra

Results & Discussion

Cold Press Method

The Hazelnut varieties show significant differences in the mass of kernel to the mass of nut. The following Table 1 and Figure 1 will clearly demonstrate the differences of kernel to nut in the varieties.

The following results were obtained by measuring the mass of the sample after cold extraction. The oil lost by mass, not recovered in the vial was used to calculate the data in Table 2, Table 3, Figure 2, and Figure 3. Any commercial process will likely recover oil more efficiently.

Table 2: Differentiation of Hazelnut Varieties by Oil Content

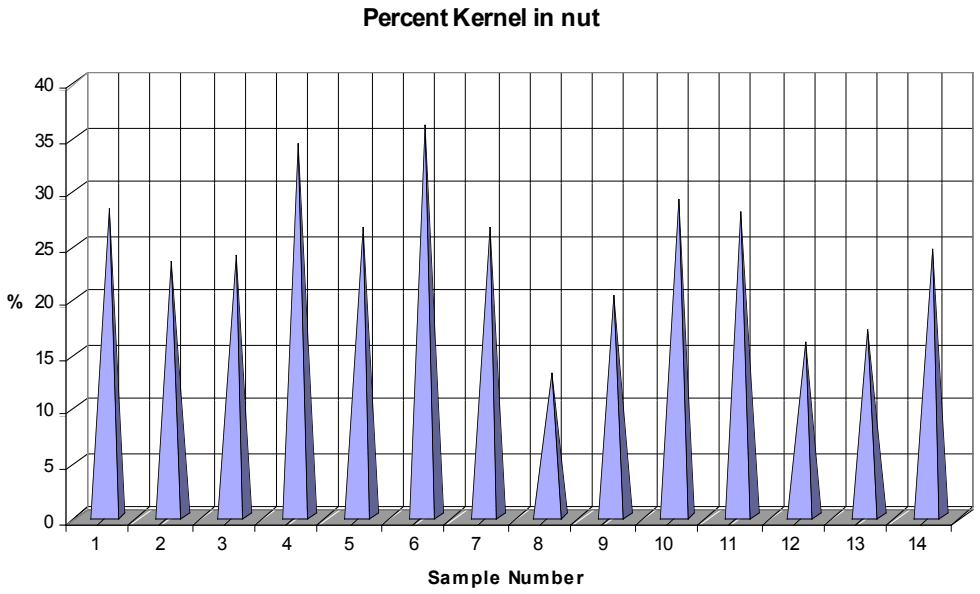
Sample#	% kernel/nut	% oil per Kernel	% oil per nut
1	28.3	8.17	2.25
2	23.46	11.78	2.76
3	23.94	21.38	5.12
4	34.27	13.4	4.59
5	26.64	8.29	2.21
6	36.03	18.49	6.66
7	26.68	14.44	3.85
8	13.35	0.25	0.03

9	20.45	12.08	2.47
10	29.14	22.91	6.68
11	28.19	6.27	1.77
12	16.01	21.25	3.4
13	17.11	15.84	2.71
14	24.69	22.68	5.6

Sum (mass of oil lost by extraction) *100% = % Oil per Kernel or Nut (g/g)
Sum (mass of kernel or nut)

Sum (kernels)*100% = % Kernels in Nut
Sum (nuts)

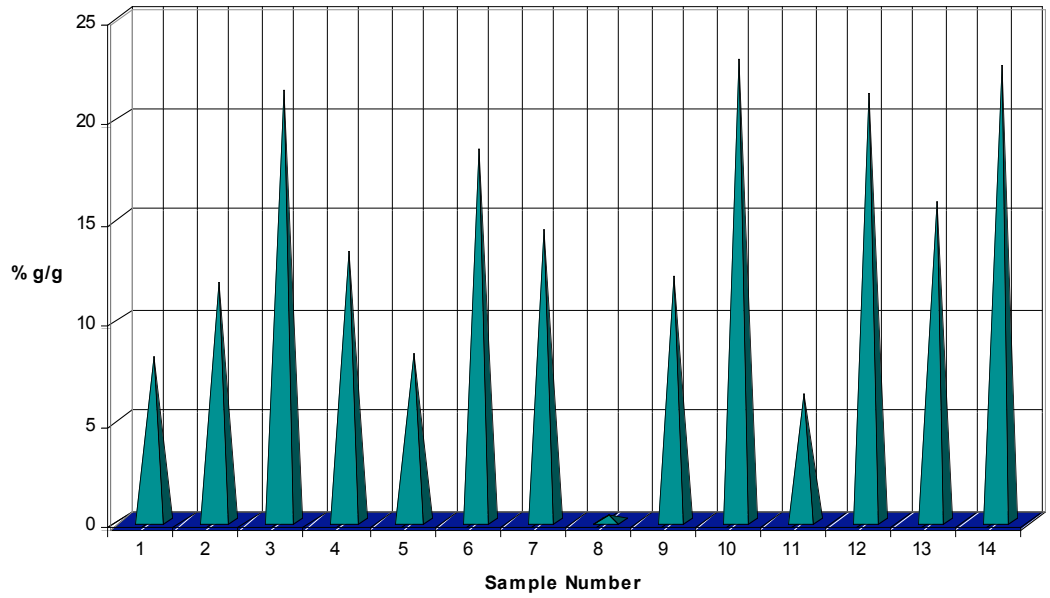
Figure 1 Differentiation of Hazelnut Varieties by Percent Kernel in Nut



The kernel is the component of interest for our discussion in Figure 1. The standard deviation of the kernel to nut percent is 6.53. The varieties with the highest values are samples 4, 6, and 10 which are 34.27, 36.03, and 29.14, respectively. The greater the mass of shell would be a benefit if the shells were to be sold at a high price. Larger kernel

to nut ratio would be a benefit if the mash was to be sold for a higher price than the shells. The samples 3, 4, and 10 would provide the most kernel available per unit weight. The lower values of percent kernel to nut suggest samples have smaller kernels and larger shells, which would likely contain less oil. The lowest values are found in sample or 8, 12, and 13. Further differentiation is needed measure oil content.

Figure 2 Differentiation of Hazelnut Oil in Kernel

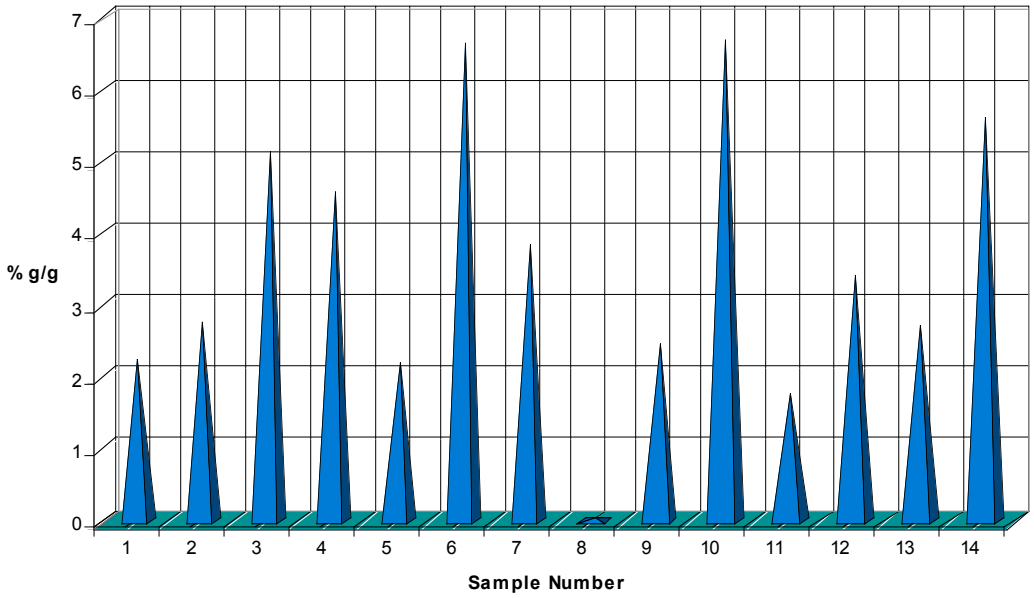


Sum (oil extracted mass)*100% = % Hazelnut oil in kernel (g/g)
Sum (kernel mass)

The oil extracted in each trial= g kernels – g mash (after oil extraction)

The standard deviation of the varieties in Figure 2 is 6.85. There is a significant difference between the varieties in terms of oil available in the kernel. Samples 3, 6, 10, 12, and 14 have the highest levels of extractible oils per kernel. These samples are effective oil producers and represent the potentially successful genetics. They produce more triacylglycerols per kernel.

Figure 3 Differentiation of Hazelnut Oil in Nut

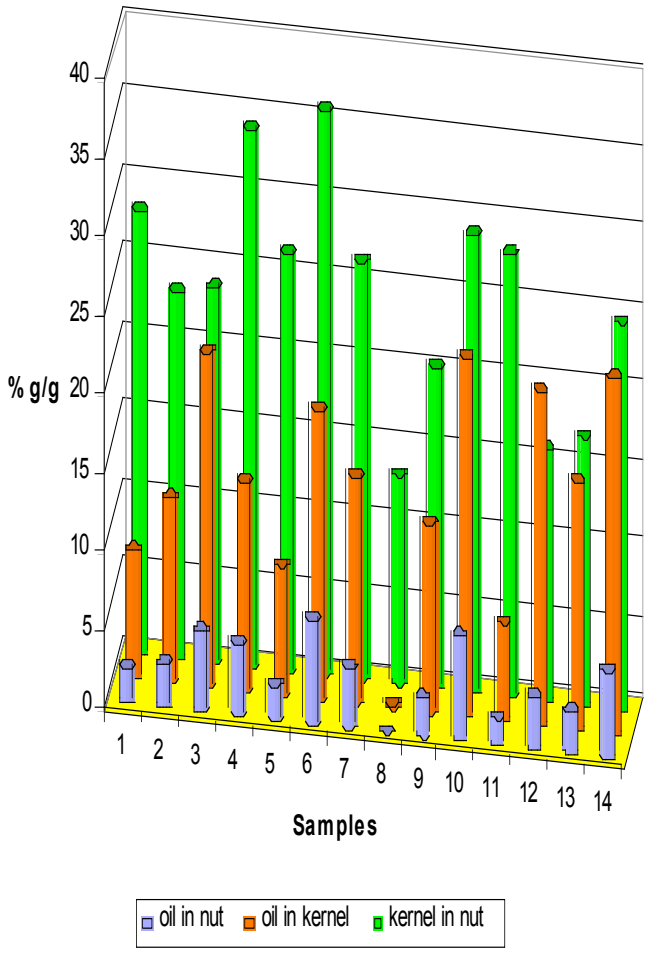


To better differentiate between the varieties, the amount of extractible oil is compared with the amount of nut in Figure 3. The advantage to this comparison is that the amount of oil a years growth produces can be approximated easily.

$$\frac{\text{Sum (oil extracted)}*100\%}{\text{Sum (mass Nuts)}} = \%\ \text{oil in nut (g/g)}$$

Samples 3, 4, 6, 10, and 14 show the highest oil per nut percentage, which are 5.12, The amount of nut needed to produce any amount of cold oil is less. Thus these samples are more sophisticated in triacylglycerol production. And likely these samples would be the best oil producers in the varieties.

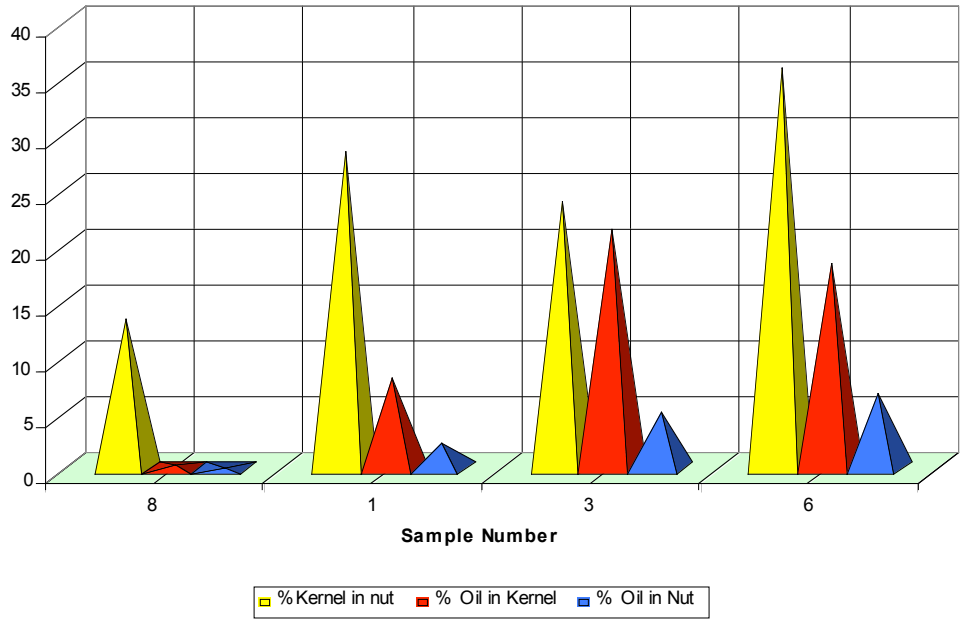
Figure 4 Differentiation of the Hazelnut Oil Content



The overall oil content from the last three Figures 1, 2, and 3 is plotted in Figure 4. When samples show higher levels in all categories, cold oil extraction is the likely route of

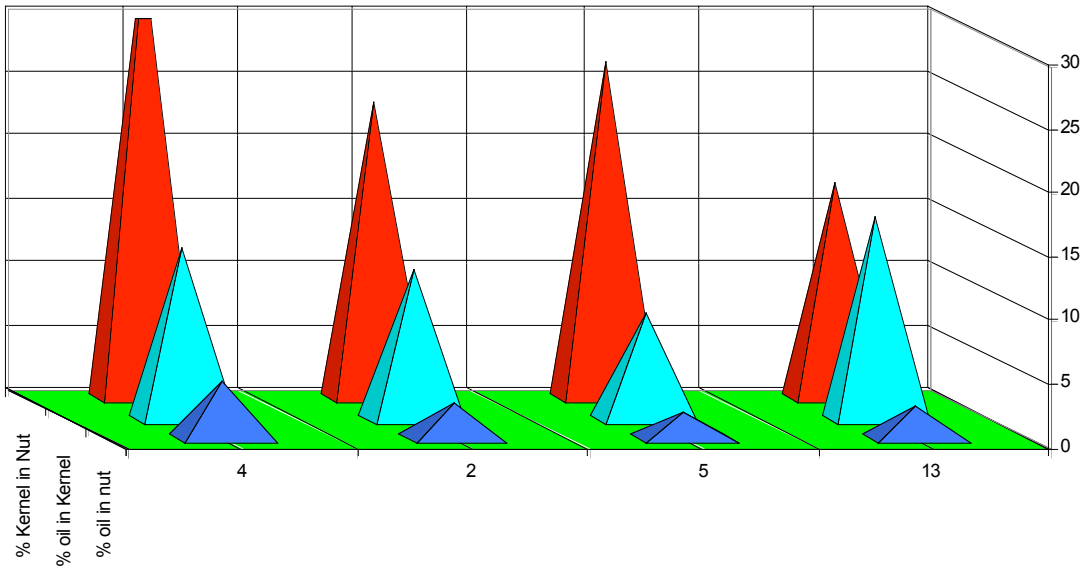
product development. They are likely to produce the highest profit in commercial production. Specifically, 3, 4, 6, 10, and 14 score the best results. They are excellent sources of oil and likely to survive the market demand which must be considered if further production is to occur. When samples show low values, commercial production of cold pressed oil is likely to as successful.

Figure 5 American Avellana Genetics



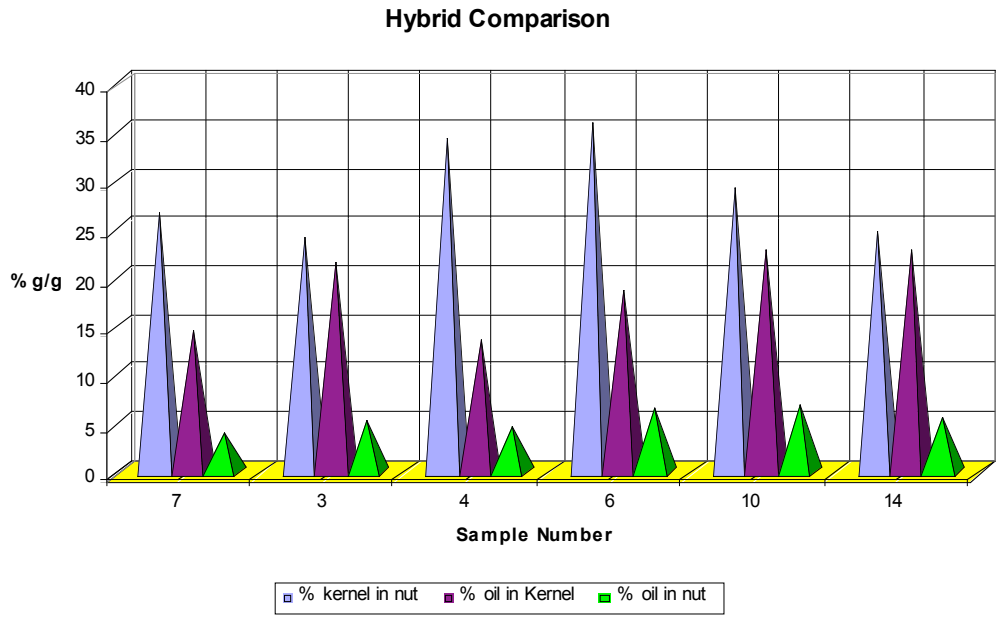
All samples in Figure 5 are Americana-avellana. Cold oils produced from sample 3 would likely be close to sample 6. Sample 6 is produced the most oil per nut and lowest relative shell mass. Sample 3 contains more oil per kernel than sample 6. Sample 3 and 6 would likely have comparable profits if demands for shell were low.

Figure 6 Americana-Avellana-Conuta Genetic



The Figure 6 demonstrates the genetic variation in Americana- Avellana- Cornuta crosses with the exception of 13. Sample 4 would harvest the most cold oil of the group, but would also produce less oil in kernel as sample 13. Sample 4 also shows lowered shell mass. Sample 4 is the best oil producer of the cornuta genetic family.

Figure 7 Hybrid Comparison



The best quality genetics in the varieties are compared in Figure 7. Samples 6 and 10 have the highest oil in nut values. Sample 3 and 14 near the best two samples and are the closest of the group. Americana-avellana produces similar oil levels in the nut as does as does a mixture of Colurna-turkish. The top three samples that produced the highest levels of oil in kernel are 3, 10, and 14. The difference between the three is small. This suggests the commercial production of the three samples is similar in potential profits. Sample 4 and 6 have the lowest ratio of shell.

Solvent Extraction

During the method refinements stage, the cold press method prevented the calculations for extracted oil to be calculated. With limited apparatus capability, the differentiation of total crude oil would be much too time consuming. Thus the results contain no real information.

Kjeldahl's Crude Protein Analysis

Table #3: Crude Protein in Hazelnut Mash

Hazelnut Mash Sample #	% Protein 5.30	% protein 6.25	% RSD
1	23.60	28.09	3.66
2	21.98	26.26	3.07
3	27.96	32.97	2.11
4	18.91	22.30	2.70
5	23.85	27.80	1.66
6	21.43	25.27	5.15
7	24.00	28.30	1.44
8	22.88	26.98	0.05
9	22.87	26.96	6.15
10	23.85	28.13	2.28
11	27.70	33.66	1.49
12	25.43	29.99	15.65
13	25.57	30.15	3.71
14	27.09	31.95	2.82

$$\frac{(\text{mL HCl Sample} - \text{mL HCl Blank}) * \text{Molarity HCL} * 14.0067\text{gN} * (5.30 \text{ or } 6.25 \text{ g Protein}) * 100\%}{\text{sample mass}}$$

Table 2 shows the amount of protein in the mash, and is visually compared in Figure 8.

Figure 8 Protein Differentiations in Hazelnut Mash

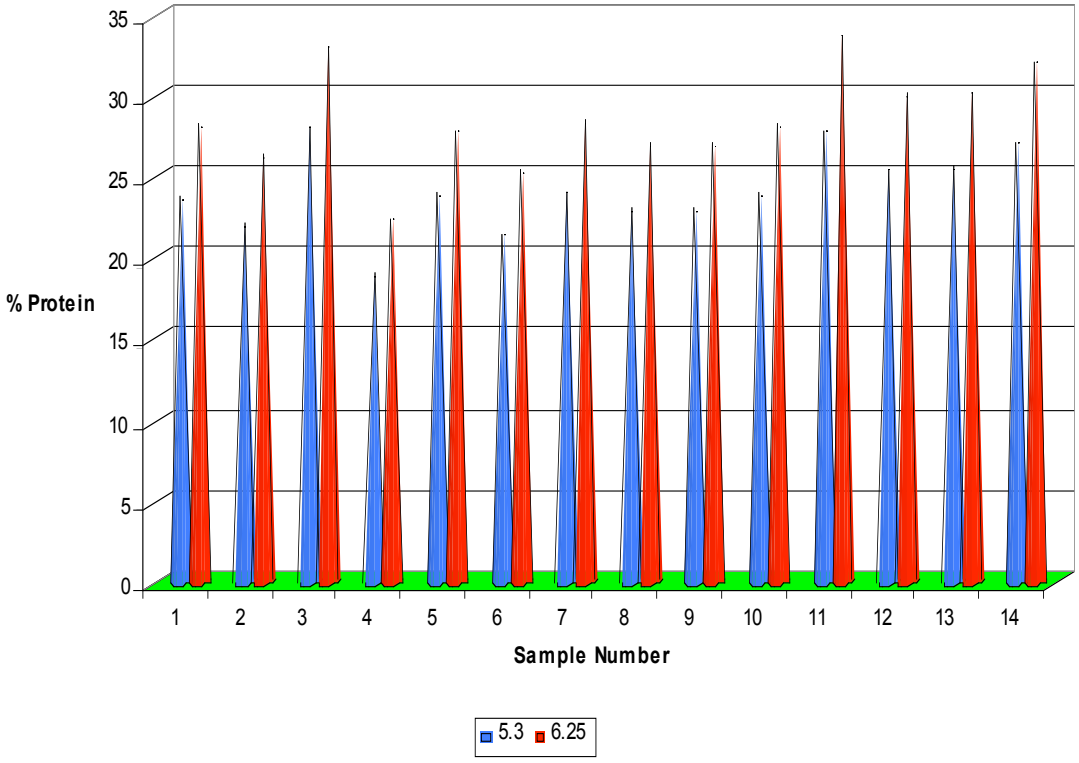


Figure 8 differentiates between the crude protein in hazelnut mash. This source of protein is not as high in comparison to other sources such as Soy meal. Yet, the potential for the hazelnut mash to be marketed and sold on protein content is not overlooked. Samples 3, 11, and 14 have the highest levels of protein from the group. More research is needed to determine which samples would provide the best sources of protein and would likely be sold for the highest value per gram.

The method was not as accurate as what initial predicted, yet results were reproducible within a reasonable range. The control ammonium chloride was used to evaluate the

method for crude protein. The recovery of ammonium chloride is calculated and measured relative to the amount of ammonium chloride from the control. The percent recovery for the 6 controls performed ranged from 95% to 101%. There was about a 5% variation which suggests the reproducibility is not the best compared to newer techniques.

Results

Calibration –

Table 4: Calibration of Fatty Acid Methyl Ester Sigma Standards

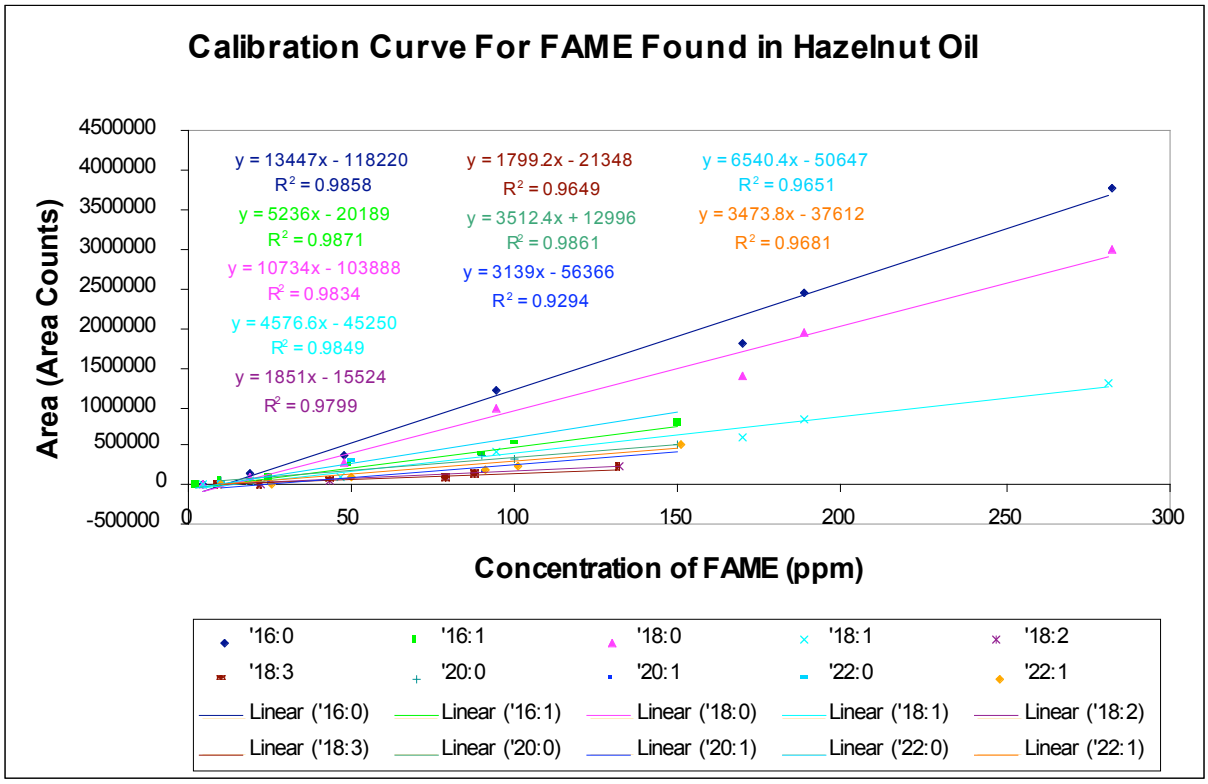
16:0		16:1		18:0		18:1	
conc.	area	conc.	area	conc.	area	conc.	area
4.7	51610	2.5	9976	4.7	37146	4.7	11444
18.8	176757	10	42775	18.8	127588	18.8	51547
47	411497	25	87720	47	311794	47	122915
94	1217444	50	258715	94	980045	94	427056
169.2	1826034	90	386350	169.2	1426407	169.2	617953
188	2487157	100	524244	188	1979022	188	847782
282	3809707	150	787269	282	3037692	282	1282795
16:0		16:1		18:0		18:1	
RF	Gap	RF	Gap	RF	Gap	RF	Gap
8755.2553	646.7128	3508.8000	481.6000	6633.9149	152.6809	2434.8936	180.3191
9401.9681	646.7128	3990.4000	287.1000	6786.5957	152.6809	2615.2128	126.6489
10792.1631	188.6879	4277.5000	15.2778	7903.4043	526.8972	2741.8617	126.6489
10980.8511	188.6879	4292.7778	15.2778	8430.3014	526.8972	3652.2045	857.2742
12951.5319	278.0266	5174.3000	68.1400	10426.0106	100.7021	4509.4787	33.6702
13229.5585	278.0266	5242.4400	6.0200	10526.7128	100.7021	4543.1489	5.7695
13509.5993	280.0408	5248.4600	6.0200	10771.9574	245.2447	4548.9184	5.7695
Q-test:	0.136026	**Q-test:**	0.276836	**Q-test:**	0.12733	**Q-test:**	0.405518
18:2		18:3		20:0		20:1	
conc.	area	conc.	area	conc.	area	conc.	area
2.2	----	2.2	----	2.5	----	2.5	----
8.8	8905	8.8	4898	10	43036	10	15138
22	21333	22	13173	25	91181	25	25388
44	71154	44	65536	50	307999	50	94893
79.2	107986	79.2	91564	90	371436	90	160962
88	151470	88	140558	100	351733	100	226642
132	238280	132	229101	150	524729	150	472880
18:2		18:3		20:0		20:1	
RF	Gap	RF	Gap	RF	Gap	RF	Gap
969.6818	42.2500	556.5909	42.1818	3498.1933	19.1367	1015.5200	498.2800
1011.9318	42.2500	598.7727	42.1818	3517.3300	19.1367	1513.8000	274.6667

1363.4596	253.6768	1156.1111	333.3434	3647.2400	129.9100	1788.4667	109.3933
1617.1364	104.1136	1489.4545	107.7955	4127.0667	176.5333	1897.8600	109.3933
1721.2500	83.9015	1597.2500	107.7955	4303.6000	176.5333	2266.4200	368.5600
1805.1515	83.9015	1735.6136	138.3636	6159.9800	1856.3800	3152.5333	886.1133
----	----	----	----	----	----	----	----
Q-test:	0.303634	**Q-test:**	0.282729	**Q-test:**	0.697419	**Q-test:**	0.41465

Table 4: Calibration of Fatty Acid Methyl Ester Sigma Standards (continued)

22:0		22:1	
conc.	area	conc.	area
2.5	9366	2.5	----
10	36219	10	20669
25	92142	25	44745
50	299010	50	150657
90	419284	90	225919
100	563478	100	284839
150	1021998	150	523859
22:0		22:1	
RF	Gap	RF	Gap
3621.9000	63.7800	1789.8000	277.1000
3685.6800	60.7200	2066.9000	277.1000
3746.4000	60.7200	2510.2111	338.1789
4658.7111	912.3111	2848.3900	164.7500
5634.7800	345.4200	3013.1400	164.7500
5980.2000	345.4200	3492.3933	479.2533
6813.3200	833.1200	----	----
Q-test:	0.285864	**Q-test:**	0.281484

Figure 9: Calibration Curve of Fatty Acid Methyl Ester Standards



Analysis of diluted hazelnut samples –

Table 5: Concentration of Fatty Acid Methyl Esters Present for Each Hazelnut Sample (Diluted 1:5)

Hazelnut Sample	Concentration (ppm)					Mass Used (mg)
	16:0	16:1	18:0	18:1	18:2	
1	95.9003	9.2032	32.8851	2004.2652	958.7931	26.9
2	20.7181		13.1417	246.5014	99.1401	17.1
3	33.8283		20.2533	721.5523	294.7685	21.2
4	55.2260	6.3413	30.1473	1370.0539	438.7615	20.2
6	72.9230	5.8606	41.9472	1996.6253	458.1268	22.8
7	84.0708	5.8046	51.3370	2170.1488	857.0016	26.7
10	65.6771	5.5485	39.1805	1856.3865	560.9863	28.5
11	62.3188	6.8858	41.0015	2016.7794	518.6411	30.9
12	37.2185	4.7912	18.6279	821.0322	267.4260	21.8
13	36.2359	5.0038	23.7514	831.9601	258.4724	23.0
14	19.5133	4.6165	12.9347	205.2455	63.0439	26.9
Hazelnut Sample	Concentration (ppm)					Mass Used (mg)
	18:3	20:0	20:1	22:0	22:1	
1				8.3011		26.9
2				8.5207		17.1
3				9.0861		21.2
4						20.2
6						22.8
7						26.7
10						28.5
11						30.9
12						21.8
13						23.0
14						26.9

Table 6: Percent Composition of Fatty Acid Methyl Esters Present for Each Hazelnut Sample (Diluted 1:5)

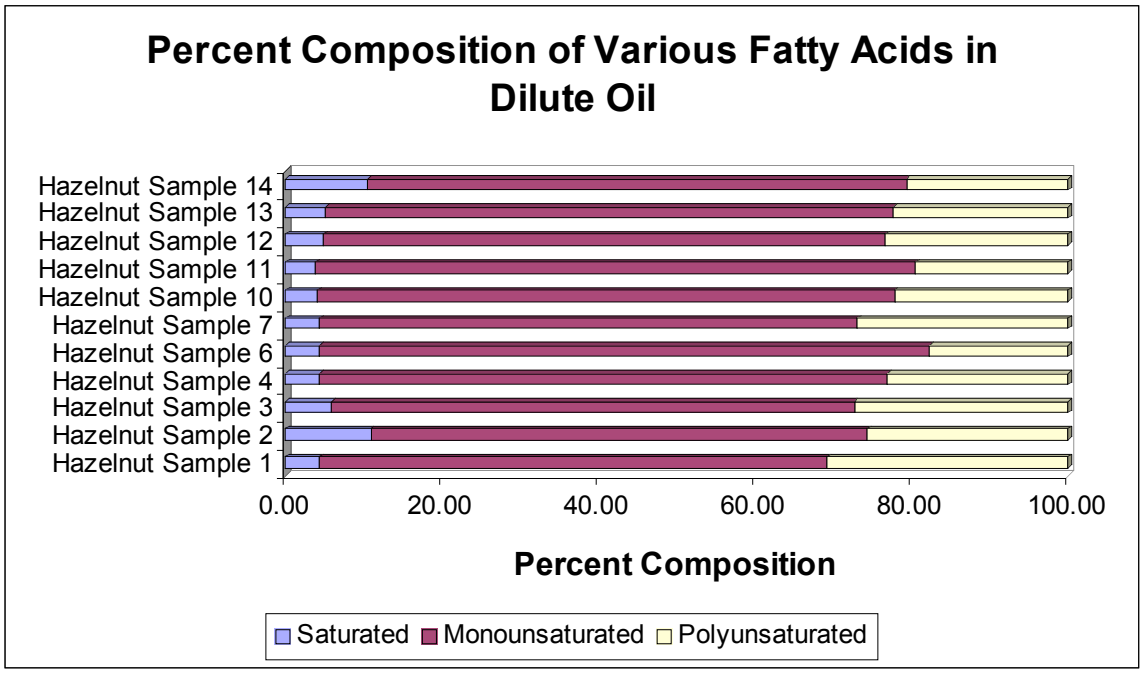
Hazelnut Sample	Percent Composition of Fatty Acids/mg of Oil				
	16:0	18:0	20:0	22:0	16:1
1	0.5733	0.1966		0.0496	0.0550
2	1.5612	0.9903		0.6421	
3	0.7391	0.4425		0.1985	
4	0.7193	0.3926			0.0826
6	0.6209	0.3572			0.0499
7	0.4969	0.3034			0.0343
10	0.4558	0.2719			0.0385
11	0.3812	0.2508			0.0421
12	0.7429	0.3718			0.0956
13	0.6818	0.4469			0.0941
14	1.1878	0.7874			0.2810
Hazelnut Sample	Percent Composition of Fatty Acids/mg of Oil				
	18:1	20:1	22:1	18:2	18:3
1	11.9813			5.7316	
2	18.5753			7.4708	
3	15.7646			6.4402	
4	17.8436			5.7144	
6	17.0009			3.9009	
7	12.8267			5.0653	
10	12.8841			3.8935	
11	12.3351			3.1721	
12	16.3877			5.3378	
13	15.6532			4.8631	
14	12.4936			3.8376	

Table 7: Percent Composition of Various Fatty Acid Types for Each Hazelnut Sample (Diluted 1:5)

Fatty Acid	% Composition of Hazelnut Samples			
	Hazelnut Sample 1	Hazelnut Sample 2	Hazelnut Sample 3	Hazelnut Sample 4
Saturated	4.3457	5.3033	4.7427	4.8616
Monounsaturated	63.8176	66.9368	71.7860	74.7007
Polyunsaturated	31.8368	27.7599	23.4713	20.4377
Fatty Acid	% Composition of Hazelnut Samples			
	Hazelnut Sample 6	Hazelnut Sample 7	Hazelnut Sample 10	Hazelnut Sample 11
Saturated	4.9770	4.6112	4.6805	4.2028
Monounsaturated	78.8352	70.7555	76.0498	78.0277
Polyunsaturated	16.1878	24.6333	19.2697	17.7694

Fatty Acid	% Composition of Hazelnut Samples		
	Hazelnut Sample 12	Hazelnut Sample 13	Hazelnut Sample 14
Saturated	4.6229	5.0256	8.0296
Monounsaturated	74.0115	74.7956	70.1379
Polyunsaturated	21.3656	20.1787	21.8325

Figure 10: Percent Composition of Various Fatty Acid Types in Each Hazelnut Sample (Dilute 1:5)



Calculations:

Standards:

(FAME 16:0)

Table 8: Original Concentration of Fatty Acid Methyl Ester Standard

Component	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
Concentration (ppm)	470.0	250.0	470.0	470.0	220.0	220.0	250.0	250.0	250.0	250.0

Standard 1: -take 1mL of Standard #3 and place in a 10mL volumetric flask

$$C_{std3}V_{std3} = C_{std1}V_{std1}$$

$$(47ppm)(1mL) = C_{std1}(10mL)$$

$$C_{std1} = [(47ppm)(1mL)]/(10mL)$$

$$C_{std1} = 4.7ppm$$

Standard 2: -take 1mL of Standard #6 and place in a 10mL volumetric flask

Standard 3: -take 1mL of FAME Stock solution and place in a 10mL volumetric flask

Standard 4: -take 1mL of FAME Stock solution and place in a 5mL volumetric flask

Standard 5: -take 3mL of Standard #7 and place in a 5mL volumetric flask

Standard 6: -take 2mL of FAME Stock solution and place in a 5mL volumetric flask

Standard 7: -take 3mL of FAME Stock solution and place in a 5mL volumetric flask

Hazelnut Sample Concentrations:

(y = Area (Area Counts); x = Concentration (ppm))

FAME 16:0 : y = 13447x - 118220

FAME 16:1 : y = 5236x - 20189

FAME 18:0 : y = 10734x - 103888

FAME 18:1 : y = 4576.6x - 45250

FAME 18:2 : y = 1851x - 15524

FAME 18:3 : y = 1799.2x - 21348

FAME 20:0 : y = 3331.1x + 45734

FAME 20:1 : y = 3139x - 56366

FAME 22:0 : y = 6540.4x - 50647

FAME 22:1 : y = 3473.8x - 37612

Dwell time:

0.35minutes x (60sec/1min) x (10^3msec/1sec) = 21,000ms

cycle time = 21,000msec/15 cycles = 1,400msec/cycle

dwell time = (1,400msec/cycle – [18msec + 4 ions x 13msec])/4 ions = 300msec

Discussion:

By using the GC-MS, we were able to analyze for various types of fatty acids, and their concentrations in different types of hazelnuts. This was done in order to determine which hazelnut had the best marketability based on its concentrations of the fatty acids.

Hazelnut samples 1 through 14 have been ranked in the following order of highest marketability to lowest marketable quality based on the percent composition of saturated, monounsaturated and polyunsaturated fats in each hazelnut oil. Referring to Table 10 – Percent Composition of Fatty Acid Methyl Esters Present for Each Hazelnut Sample and Figure 11- Percent Composition of Fatty Acid Methyl Esters in Each Hazelnut Sample , the following tabulated results were obtained:

Table 9: Tabulation of Hazelnuts ranked in order of desirability of % Fats in Oil

% Saturated Fats		% Monounsaturated Fats		% Polyunsaturated Fats	
Undiluted	Diluted	Undiluted	Diluted	Undiluted	Diluted
11	11	6	6	1	1
1	10	11	11	2	3
7	7	10	10	7	7
12	1	13	13	3	2
10	6	4	4	14	12
3	4	12	12	12	4
4	12	3	14	4	13
6	3	7	7	13	10
13	13	14	3	10	14
2	14	2	1	11	11
14	2	1	2	6	6

*** A low concentration of saturated fatty acids is desirable, while a high concentration of monounsaturated and polyunsaturated fatty acids is desirable

*** Numbers in Table 11 represent the Hazelnut sample number

It is important to know the percent fatty acid of the oil and the percent fatty acid of the kernel, both for different reasons. The percent fatty acid per milligram of oil is important to know for cooking oil producers and for them to market their product. It is important to know the percent fatty acids per kernel for manufacturers that press the hazelnuts and sell the crude oil to other manufactures. This is because manufacturers don't want to continually harvest hazelnuts that produce minute amounts of oil per

kernel. Manufacturer's want to obtain the greatest possible amount of oil in the least amount of time, efficiency is important.

Table 10: Percent Composition of Fatty Acid Methyl Esters Present for Each Hazelnut Sample

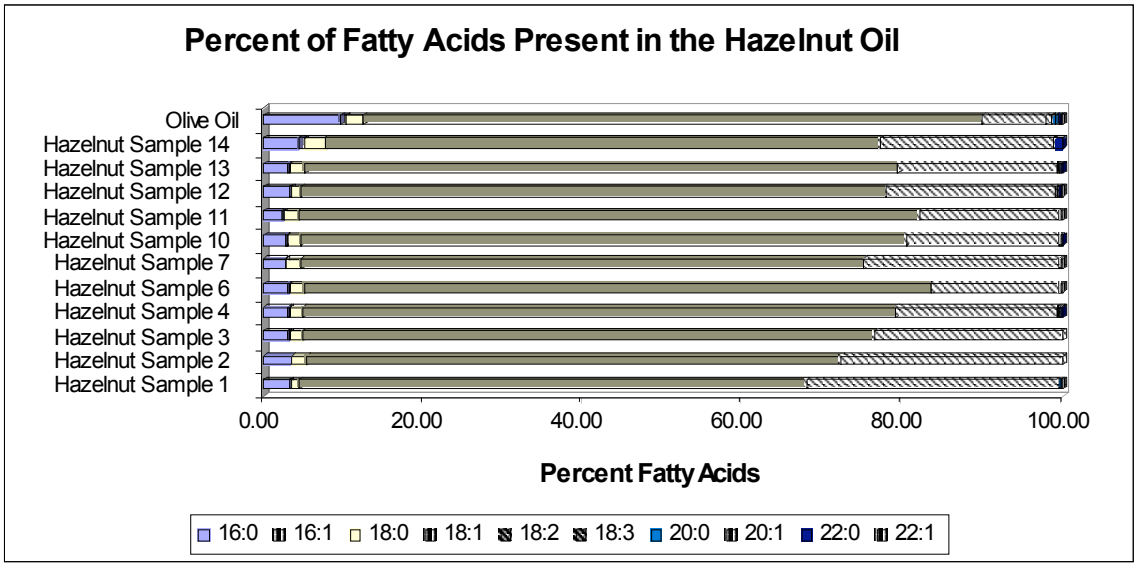
	Percent Composition of Fatty Acids in Oil				
	16:0	16:1	18:0	18:1	18:2
Hazelnut Sample 1	3.2596	0.2043	1.0487	63.3649	31.6589
Hazelnut Sample 2	3.6508		1.6525	66.9368	27.7599
Hazelnut Sample 3	3.1482	0.2052	1.5945	71.5807	23.4713
Hazelnut Sample 4	3.1576	0.2032	1.5692	74.1852	20.1714
Hazelnut Sample 6	3.1802	0.1782	1.7968	78.3848	15.9479
Hazelnut Sample 7	2.7770	0.1632	1.8342	70.3296	24.4237
Hazelnut Sample 10	2.8401	0.1595	1.7511	75.6848	19.0315
Hazelnut Sample 11	2.4509	0.1568	1.7519	77.6240	17.5530
Hazelnut Sample 12	3.2871	0.2222	1.1980	73.2043	21.1219
Hazelnut Sample 13	3.0352	0.2185	1.8521	74.2702	19.8743
Hazelnut Sample 14	4.3697	0.7045	2.6565	69.4334	21.8325
Olive Oil	9.6066	0.6424	2.2287	77.3867	8.1950
	Percent Composition of Fatty Acids in Oil				
	18:3	20:0	20:1	22:0	22:1
Hazelnut Sample 1	0.1778	0.0373	0.2484		
Hazelnut Sample 2					
Hazelnut Sample 3					
Hazelnut Sample 4	0.2664		0.3123	0.1347	
Hazelnut Sample 6	0.2399		0.2723		
Hazelnut Sample 7	0.2097		0.2627		
Hazelnut Sample 10	0.2382		0.2055	0.0893	
Hazelnut Sample 11	0.2164		0.2470		
Hazelnut Sample 12	0.2438		0.3744	0.1377	0.2105
Hazelnut Sample 13	0.3045		0.3069	0.1383	
Hazelnut Sample 14				1.0034	
Olive Oil	0.6528	0.4756	0.4176	0.1961	0.1985

Referring to Table 10: Percent Composition of Fatty Acid Methyl Ester Present for each Hazelnut Sample, it is important to note that 22:1, commonly known as Erucic Acid, was only detected in trace amounts in Hazelnut sample 12 and Olive oil There was found to be 0.21% of 22:1 in Hazelnut sample 12 and 0.20% in Olive oil. Erucic Acid is considered a possible carcinogen and Hazelnut Oil sample 12, becomes less marketable, compared to the other Hazelnut samples.

Table 11: Percent Composition of Various Fatty Acid Types for Each Hazelnut Sample

Fatty Acid	% Composition of Hazelnut Samples			
	Hazelnut Sample 1	Hazelnut Sample 2	Hazelnut Sample 3	Hazelnut Sample 4
Saturated	4.3457	5.3033	4.7427	4.8616
Monounsaturated	63.8176	66.9368	71.7860	74.7007
Polyunsaturated	31.8368	27.7599	23.4713	20.4377
Fatty Acid	% Composition of Hazelnut Samples			
	Hazelnut Sample 6	Hazelnut Sample 7	Hazelnut Sample 10	Hazelnut Sample 11
Saturated	4.9770	4.6112	4.6805	4.2028
Monounsaturated	78.8352	70.7555	76.0498	78.0277
Polyunsaturated	16.1878	24.6333	19.2697	17.7694
Fatty Acid	% Composition of Hazelnut Samples			
	Hazelnut Sample 12	Hazelnut Sample 13	Hazelnut Sample 14	Olive Oil
Saturated	4.6229	5.0256	8.0296	12.5070
Monounsaturated	74.0115	74.7956	70.1379	78.6452
Polyunsaturated	21.3656	20.1787	21.8325	8.8477

Figure 11: Percent Composition of Fatty Acid Methyl Esters in Each Hazelnut Sample

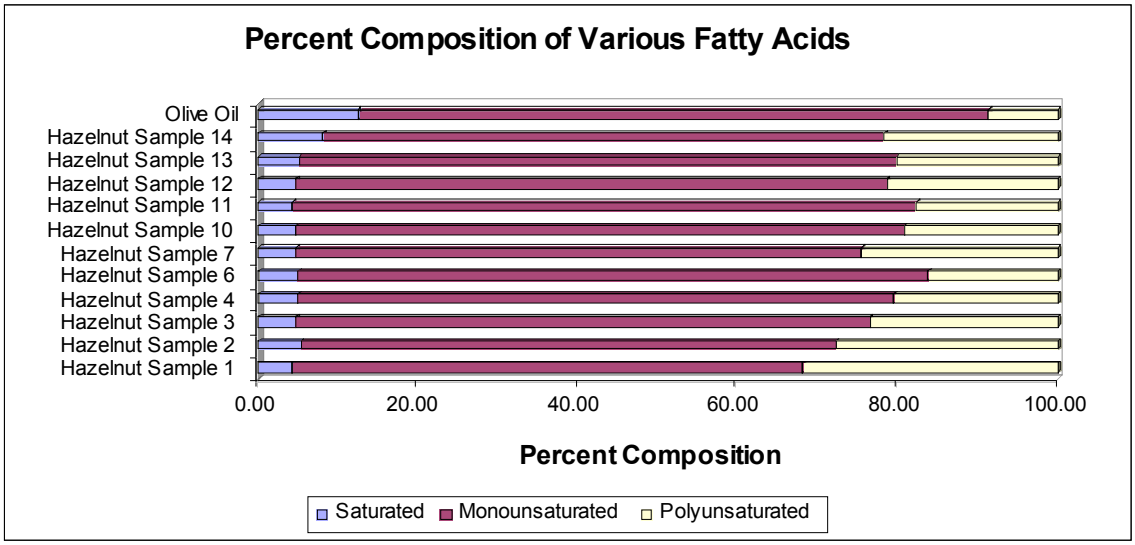


Referring to Figure 11- Percent Composition of Fatty Acid Methyl Esters in each Hazelnut Sample, the percentage of 16:0 (palmitic acid), was found to be less in all of the hazelnut samples, when compared to olive oil. As well, the percentage of 16:1 (Palmitoleic acid) was less in all hazelnut oils samples when compared to that of olive oil, having 0.64% Palmitoleic acid.

The green bar in Figure 11- Percent Composition of Fatty Acid Methyl Esters in each Hazelnut Sample, represents the percentage of 18:1 (oleic acid), all hazelnut

samples including olive oil contain approximately the same percentage of oleic acid, +/- 10%. The maroon bar represents the amount of 18:2 (Linoleic acid), and compared to 8.2% of olive oil, every hazelnut sample contained a higher percentage of Linoleic acid.

Figure 12: Percent Composition of Various Fatty Acid Types in Each Hazelnut Sample



Referring to Figure 12- Percent Composition of Various Fatty Acids in Each Hazelnut Sample, hazelnut samples 1 – 14 were compared to olive oil for % mono and polyunsaturated and saturated fats. The light purple bar represents the percentage of saturated fats. Olive oil clearly contains more saturated fats than any of the hazelnut samples analyzed. Saturated fats increase heart risk and cholesterol, therefore the Badgersett hazelnut oil is more marketable because there is less saturated fats.

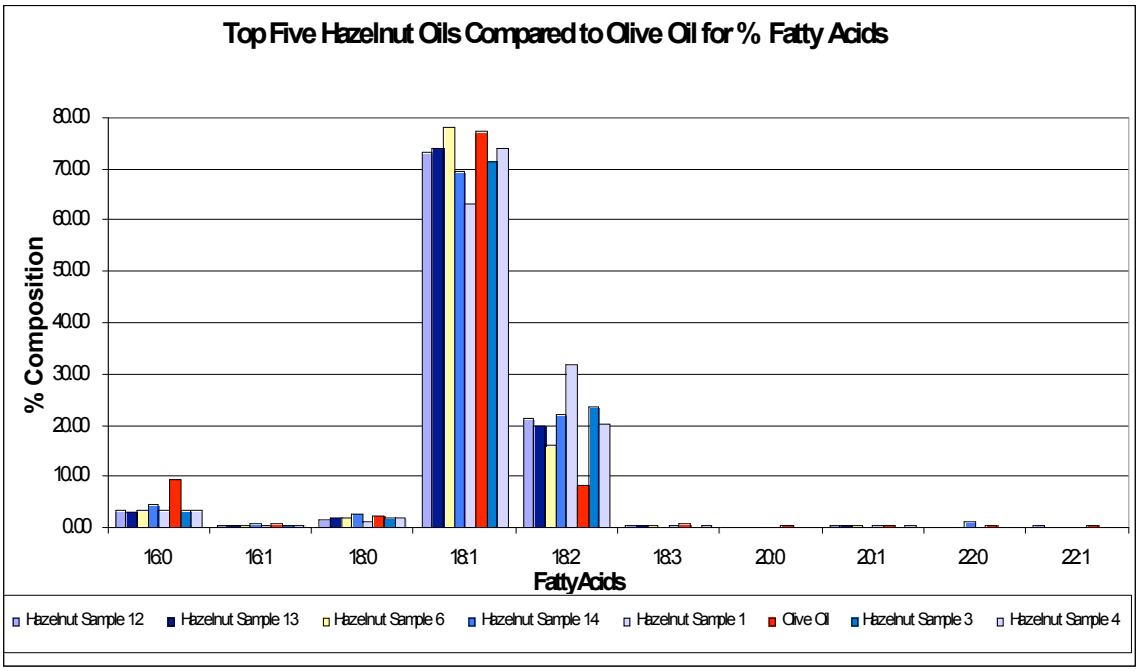
Referring to the maroon bar of Figure 12- Percent Composition of Various Fatty Acids in Each Hazelnut Sample, this represents the mono-unsaturated fats detected in the hazelnuts samples as well as olive oil. The percentage of mono-unsaturated is consistent throughout hazelnut samples as well as olive oil, +/- 10%.

The yellow bar in Figure 12- Percent Composition of Various Fatty Acids in Each Hazelnut Sample, represents the percentage of poly-unsaturated fats, also known as 18:2 and 18:3, the essential fatty acids. Olive oil contained 8.8% poly-unsaturated fats and all of the hazelnut samples contained at least two times this amount. The hazelnut oil

samples have a high marketable quality because the oil contains more essential fatty acids than olive oil.

All of the hazelnut samples were ranked in decreasing order for % of each fatty acid and for % fats (mono and poly unsaturated and saturated). After tabulation it was determined that hazelnut samples 1, 7, 10, 11, and 12 were the top five hazelnut samples, and these samples were compared to olive oil. Hazelnut sample 3 was also included because it is a hybrid sample and analysts wanted to determine if the hybrid produced quality results compared to olive oil. Also, hazelnut sample 4 was included in the comparison because it was considered to be a likely hazelnut to go into commercial production.

Figure 13: Comparison of Top Five Hazelnut Samples to Olive Oil for Percent Composition of Fatty Acid Methyl Esters



Referring to Figure 13- Composition of Top Five Hazelnut Samples Compared to Olive Oil for Percentage of Fatty Acid Methyl Esters, the top five hazelnut samples and hazelnut samples 3 and 4 were compared to olive oil for % of each fatty acid.

In Figure 13- Composition of Top Five Hazelnut Samples Compared to Olive Oil for Percentage of Fatty Acid Methyl Esters. The top five hazelnut samples were chosen

by ranking all the hazelnut samples for each fatty acid in increasing or decreasing order of quantification results, compared to that of olive oil. For example, for 16:0, Palmitic Acid, all the hazelnut samples were ranked in decreasing order of percentage of Palmitic Acid. For Palmitic Acid, the greater the percentage, the more desirable the hazelnut sample.

The red bar represents olive oil, and by examining Palmitic acid (16:0), it is obvious that the all hazelnut samples (1, 3, 4, 7, 10, 11 & 12) contain less Palmitic acid saturated fat.

The 18:0 column (Stearic acid) displays that the top five hazelnut samples as well as hazelnut samples 3 and 4, contain approximately the same % of Stearic acid. Referring to the column of 18:1 (oleic acid) approximately all hazelnut samples including olive oil, contain between 65% - 75% oleic acid.

Referring to the 18:2 column (Linoleic acid) of Figure 13- Composition of Top Five Hazelnut Samples Compared to Olive Oil for Percentage of Fatty Acid Methyl Esters, the top five-hazelnut sample contained a higher percentage of Linoleic acid, compared to olive oil. This results in the hazelnut samples being marketable as compared to olive oil.

Lastly, referring to Figure 13- Composition of Top Five Hazelnut Samples Compared to Olive Oil for Percentage of Fatty Acid Methyl Esters, 16:1, 18:3, 2-:0, 20:1, 22:0 and 22:1 fatty acid were detected in trace amounts and are therefore were not compared.

Figure 14: Comparison of Top Five Hazelnut Samples to Olive Oil for Percent Composition of Various Fatty Acid Types

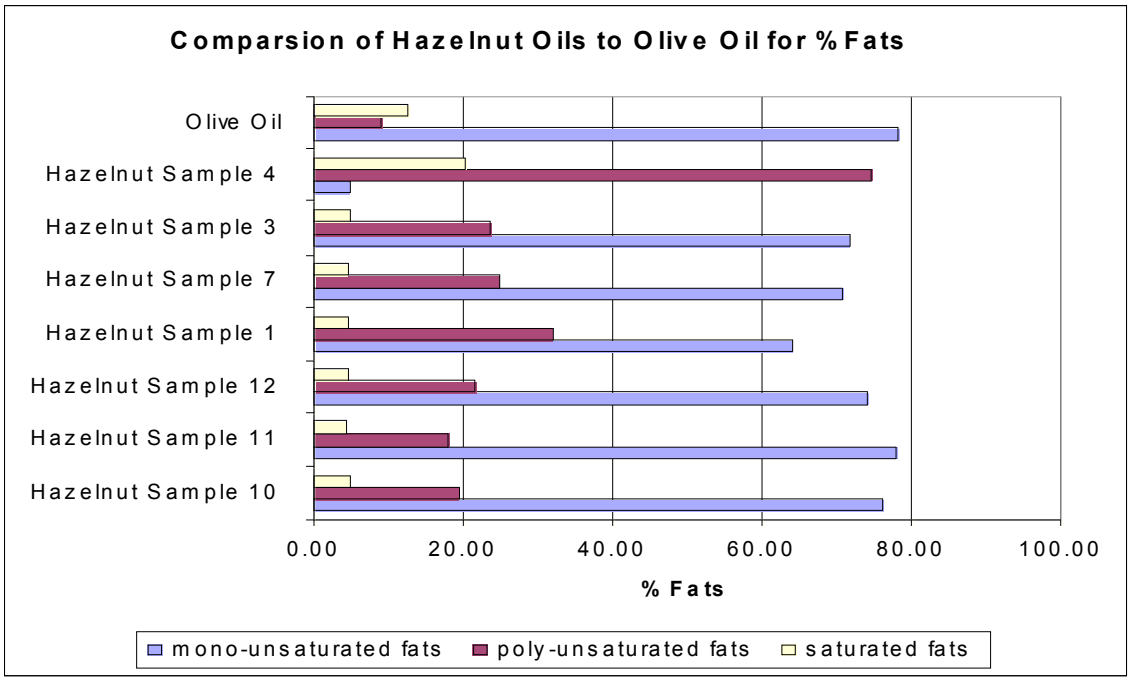


Figure 14- Comparison of Top Five Hazelnut Samples to Olive Oil for Percentage of Various Fatty Acid Types, displays the relationship of % mono and poly unsaturated fats and saturated fats of the top five hazelnut samples, including hazelnut samples 3 and 4, compared to olive oil.

The yellow bar on Figure 14- Comparison of Top Five Hazelnut Samples to Olive Oil for Percentage of Various Fatty Acid Types, represents the percentage of saturated fats quantified in each hazelnut sample as well as olive oil. The top five hazelnut samples (1, 7, 10, 11, 12) all contain less saturated fats than olive oil. Also the hybrid, hazelnut sample 3, contains less saturated fats than olive oil. Hazelnut sample 4, ("most likely to go into commercial production"), contained more saturated fat than the olive oil sampled.

The maroon bar represents the percentage of polyunsaturated fats, and the olive oil contains less poly-unsaturated fats than any hazelnut sample. For all the hazelnut oils, this is considered a major advantage because the higher the percentage of poly-unsaturated fats the more essential fatty acids. Therefore, this increases the marketability of the hazelnut oil samples over olive oil.