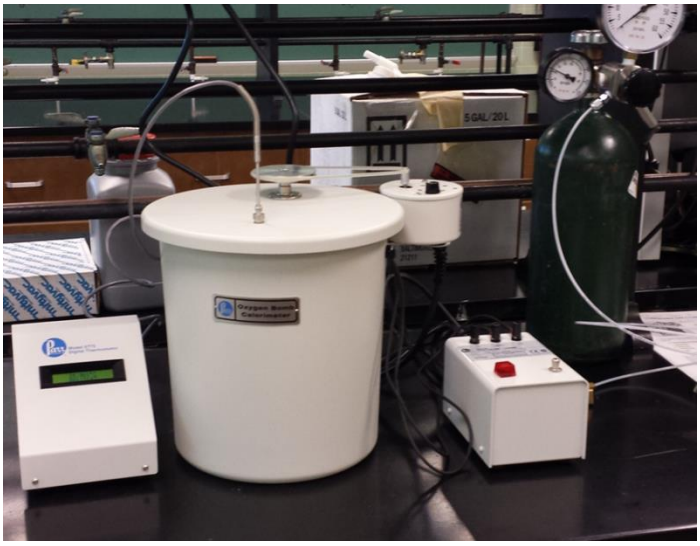
Determination of the Relative Ratio of Unsaturated Fats to Saturated Fats in Nuts Using Bomb Calorimetry



by Tara Stacey

Advisor Dr. Ozturk

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Tara Stacey

Chemistry Department

McKendree University

tlstacey@mckendree.edu

Abstract

Unsaturated fats are recommended for healthy dietary consumption as opposed to saturated fats. Conventional methods for the measurement of different fatty acid contents in natural products are HPLC/MS and GC/MS which are costly and require high levels of maintenance. The purpose of this research project was to introduce a new unconventional method which is simple and cost-effective for the comparison of the relative amounts of unsaturated and saturated fats in various types of nuts. The ten different types of tree nuts explored during this project were almonds, Brazil nuts, cashews, walnuts, hazelnuts, macadamias, pecans, pine nuts, peanuts, and pistachios. The fats and oils from these nuts were extracted through a modified Bligh-Dyer method and then combusted in a bomb calorimeter. Butyric, decanoic, myristic, palmitic, stearic, oleic, linoleic, behenic, and erucic acids, and DHA extracted from fish oil gel caps were used as standards for different chain lengths and saturation levels. Employing bomb calorimetry, the heat of combustion values were determined and used to estimate the relative amounts of unsaturated and saturated fats.

1. Introduction

The purpose of this research project was to introduce a new method which is both simple and cost-effective for the determination of the relative amounts of unsaturated fats and saturated fats in various types of nuts. The 10 different types of tree nuts explored during this project were almonds, walnuts, Brazil nuts, cashews, peanuts, hazelnuts, macadamias, pecans, pine nuts, and pistachios. Through bomb calorimetry, the heat of combustion values were used to estimate the relative amounts of unsaturated and saturated fats. The results relate directly to human health due to the fact that while dietary consumption of unsaturated fats is recommended, saturated fats are considered harmful in diet.

Triacylglycerols, diacelylglycerols, monoacylglycerols, free fatty acids, and other minute components make up the total lipid content of tree nut oils (Miraliakbari & Shahidi, 2008). The total lipid content is relatively high in tree nuts and could range anywhere from 45-75% for different nuts. Different kinds of nuts consist of fats with different contents of unsaturated and saturated fatty acid chains. In one study, it was determined that Brazil nuts, cashews, macadamias, and pistachios had higher amounts of saturated fats, respectively 24.5%, 20.9%, 17.1%, and 13.3% (Robbins et al., 2011). The common fatty acids found in lipids of these nuts are oleic acid ($C_{18:1}$), palmitoleic acid ($C_{16:1}$), linoleic acid ($C_{18:2}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and/or linolenic acid ($C_{18:3}$) (Moayedi et al, 2011; Robbins et al, 2011). Oleic acid, a monounsaturated omega-9 fatty acid, is the most abundant fatty acid in most kinds of nuts (Miraliakbari & Shahidi, 2008; Primorac & Mandic, 2000).

Saturated fatty acids with no double bonds have carbon atoms at their most reduced state, while unsaturated fats with double bonds have carbon atoms at higher oxidation states. In general, we would expect lower heat of combustion when unsaturated fats undergo complete oxidation compared to the saturated fats. Fats, overall, typically provide about 9.0 kcal per gram of metabolic energy (Merten, 1970) which can be measured using bomb calorimetry. Within the bomb calorimeter, the sample is burned in oxygen gas in a stainless steel bomb. This method was chosen because the heat of combustion is expected to depend on the hydrogen to carbon ratio of fatty acids and a decrease in this ratio is directly related to the degree of unsaturation in the fatty acid chains. Fatty acids with higher ratio of hydrogen to carbon have lower degrees of unsaturation than those with a lower H/C ratio and are expected to have higher heats of combustion. This is in agreement with the report that longer-chain and saturated fatty acids have higher kcal per gram (Merten, 1970). Although no prior data was recorded by bomb calorimetry to compare combustion heats of fats in nuts, other methods were utilized to test the content of unsaturated and saturated fats. One such experiment was the qualitative analysis done on palm olein-olive oil blend to compare the fatty acid composition of palm olein oil and olive oil. Gas chromatography was used to show the relationship that the addition of olive oil to palm oil resulted in an increase in unsaturated fat content.

The first step in fat analysis from any source requires the extraction of fat from the source mixture. There are different solvents that can be used for extraction of oils from nuts. Miraliakbari and Shahidi (3) investigated two solvents, hexane and a chloroform/ methanol mixture, for almonds, Brazil nuts, hazelnuts, pecans, pines, pistachios, and walnuts. They concluded that the chloroform/ methanol mixture yielded a higher amount of oil extraction. Centrifugation, filtration, and evaporation were part of the fat extraction process.

In this project, we tested the hypothesis that it should be possible to estimate the relative content of unsaturated versus saturated fats based on the differences in their heats of combustion using the bomb calorimetry technique. Oils from ten different kinds of nuts: almonds, walnuts, Brazil nuts, cashews, peanuts, hazelnuts, macadamias, pecans, pine nuts, and pistachios were extracted and burned in a bomb calorimeter to compare their relative heats of combustion. Nine different fatty acids were utilized as standards (butyric, decanoic, myristic, palmitic, stearic, oleic, linoleic, behenic, and erucic acid) for this comparison. Docosahexaenoic acid, or DHA, was also used as a standard, however, this fatty acid had to be extracted from fish oil softgels. Of these fatty acids, butyric, decanoic, myristic, palmitic, stearic, and behenic acids served as standards for chain length, whereas stearic, oleic, linoleic, behenic, erucic, and docosahexaenoic acids served as standards for degrees of unsaturation. To compensate for the effect of chain length on the heat of combustion, we normalized our results by calculating the degree of unsaturation per carbon unit for the nut samples.

2. Materials

The pure standards and other chemicals were purchased from Sigma-Aldrich. The different kinds of nuts were bought fresh, raw, and air-sealed from Nuts.com. Both the nitrogen and oxygen gas cylinders were acquired from CeeKay Supply, Inc. The bomb calorimeter (Parr Model: 1341 Oxygen bomb calorimeter, 1108 Oxygen combustion bomb, and 6775 Digital thermometer) and the pellet press were obtained from Parr Instrument Company. All other materials were provided by McKendree University's Science Department.

3. Procedure

Fat extraction

The nuts were stored at -40°C in nitrogen atmosphere to prevent oxidation. Extractions of the fatty acids from these nuts entailed the usage of chloroform, methanol, and anhydrous sodium sulfate. Butyric, decanoic, myristic, palmitic, stearic, oleic, linoleic, behenic, erucic, and docosahexaenoic acids were used for standardization; therefore the heats of combustion of these fatty acids were measured in their pure form, except for docosahexaenoic acid (DHA).

To begin the procedure, each of the previously frozen nut samples were crushed using a Ninja food processor and, if needed, blended into a fine powder using a mortar and pestle. 100 mL of chloroform was then added to 10 grams of each of the powdered nuts and homogenized with the assistance of a vortexer for at least 3 minutes. A Whatman #1 filter, along with a Buchner funnel and suction, was used for filtration of the mixtures. This was repeated two more times except with 100mL of a 1:1 volume ratio of chloroform/ methanol, and all three filtrations were combined into one mixture for each nut. An evaporator at 40°C was employed to remove the solvent. Chloroform then was used again in re-dissolving the oil which was followed by filtering the oil through a layer of anhydrous sodium sulfate to remove the residual water in the mixture. At 40°C, an evaporator was again utilized to separate the layers and remove the remaining solvent. The extracted oil was then transferred to a capped vial and stored at -80°C in nitrogen gas until ready for use.

DHA extraction

Docosahexaenoic acid, or DHA, was extracted from Mason's Natural DHA (200 mg per serving) Omega 3 softgels. Each softgel contained 100mg of DHA, as well as the following ingredients: gelatin, vegetable glycerin, and purified water. The fish oil in each softgel weighed approximately 0.3800±0.0100 g. Seven softgels were combined per sample in order to achieve enough DHA for combustion (>0.500g). Glycerin was evaporated from the sample using a high temperature oil bath. The evaporation process was done over many days for several hours (typically more than 4 hours) each day under nitrogen atmosphere to prevent oxidation. The temperature was monitored throughout the process with the highest temperature reaching 250°C. Evaporation was continued until the samples had a noticeable reduction in volume and a slight darkening in color. After each evaporation, the samples were capped with nitrogen gas and stored at -40°C until next evaporation or until ready to be combusted.

Combustion of standards and nut oils

A bomb calorimeter was used to determine the heat of combustion of the standards and the nut oils. First, the sample and an iron wire 10-12 centimeters in length were precisely weighed. The weighed solid samples were made into a pellet using a pellet press. Next, the pellet or liquid sample was placed into the center of the sample pan (capsule) and installed in the bomb, along with the wire. The wire was then maneuvered to touch the sample, but not the capsule. The bomb was then carefully assembled and the cap screwed on by hand-tightening. After the oxygen flow tube was attached to the bomb, it was slowly filled with oxygen gas and then released to flush out any atmospheric nitrogen gas that was already in the bomb. The bomb was filled once more with 30 atmospheres of oxygen gas. Two liters of distilled water was measured precisely with a 1.000-liter volumetric flask twice and poured into the stainless steel pail. The pail was placed in the calorimeter. The bomb was then positioned in the

pail while also affixing the electrical leads to it. The calorimeter lid was put on, and the stirrer and digital thermometer were powered on. The water bath temperature was observed and recorded every 30 seconds for 5 minutes or until the temperature reached thermal equilibrium by use of the digital thermometer with a built-in timer. After thermal equilibrium was attained, the bomb was ignited while recording the temperature and time every 10 seconds for 6 minutes followed by 30 second interval recordings for a total time of 15 minutes. After the run was completed, the unburned iron wire pieces found in the bomb were weighed to the tenth of a milligram. Any soot globules found in the capsule were crushed and deemed as burned, whereas those that didn't crush were weighed precisely and counted as unburned product.

The heat capacity of the calorimeter was found using the benzoic acid standard. Three trials were run and a fourth trial was done to confirm the calculated heat capacity of the calorimeter against the actual specific heat of combustion of benzoic acid. Initially there were issues after each bombing due to condensation and minor soot volume on the capsule where possible product was contained. This issue was resolved by weighing the unburned iron wire pieces separately into a weigh boat, and also weighing the capsule for condensation/soot comparison between each weighing.

The fatty acid standards were run at least 3 times, and an average heat of combustion was determined for each. The fatty acid standards for chain length consisted of butyric ($C_{4:0}$), decanoic ($C_{10:0}$), myristic ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and behenic acid ($C_{22:0}$) while the fatty acid standards for degree-of-unsaturation were stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), behenic acid ($C_{22:0}$), erucic acid ($C_{22:1}$), and DHA ($C_{22:6}$). The extracted nut oils and the extracted DHA were run at least 3 times as well, and an average heat of combustion was determined for each. The burning of pine nut oil, walnut oil, and almond oil in the bomb calorimeter resulted in minute amounts of white residue which was assumed to be potassium oxide since these nuts are high in potassium content.

4. Data and Results

Table 1: Heat capacity data for bomb calorimeter

Trial	Mass of capsule (g)	Mass of benzoic acid pellet (g)	Mass of iron wire (g)	Mass of unburned iron wire (g)	Change in temperature (°C)	Heat capacity of calorimeter (kJ/°C)
1	9.5313	1.0615	0.0178	0.0014	2.761	10.154
2	9.5316	1.0462	0.0171	0.0009	2.723	10.147
3	9.5317	1.0572	0.0171	0.0000	2.752	10.146
4	9.5321	1.0082	0.0165	0.0005	2.634	10.109

Average heat capacity of calorimeter for trials 1-3: **10.15 kJ/°C**

Figure 1: Calorimeter heat capacity determination for Trial 1

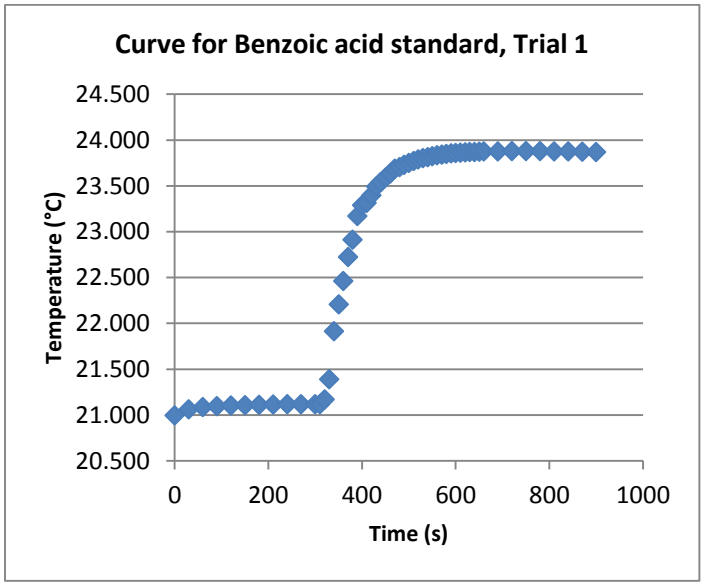


Table 2: Measured heats of combustion for fatty acid standards

Fatty Acid Standard for Chain Length	Average Heat of Combustion (kJ/g)
Butyric acid ($C_{4:0}$)	24.625
Decanoic acid ($C_{10:0}$)	35.264
Myristic acid ($C_{14:0}$)	37.770
Palmitic acid ($C_{16:0}$)	38.905
Stearic acid ($C_{18:0}$)	39.422
Behenic acid ($C_{22:0}$)	40.623
Fatty Acid Standard for Degrees of Unsaturation	**Average Heat of Combustion (kJ/g)**
Stearic acid ($C_{18:0}$)	39.422
Oleic acid ($C_{18:1}$)	39.347
Linoleic acid ($C_{18:2}$)	38.927
Behenic acid ($C_{22:0}$)	40.623
Erucic acid ($C_{22:1}$)	40.392
Docosahexaenoic acid (DHA) ($C_{22:6}$)	38.334

Figure 2: Plot of heats of combustion vs. chain length for standards

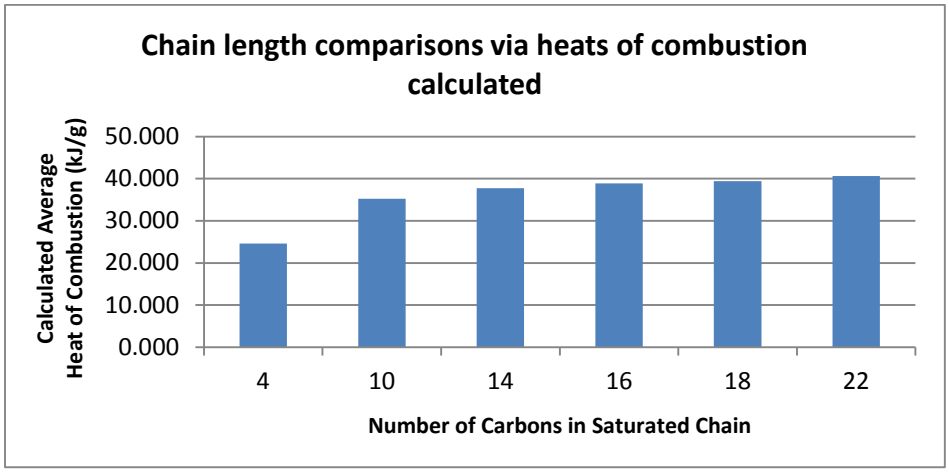


Figure 3: Plot of heats of combustion vs. degrees of unsaturation for standards

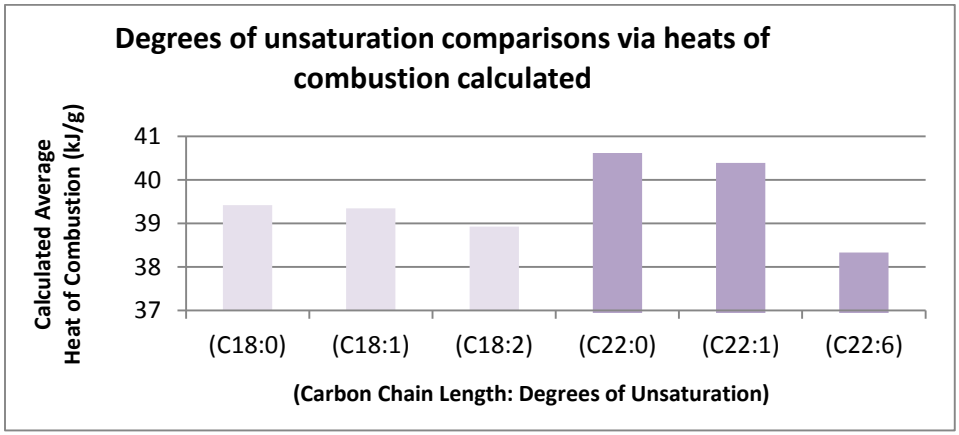


Figure 4: 3-D view of standards heats of combustion in relation to chain length and double bonds

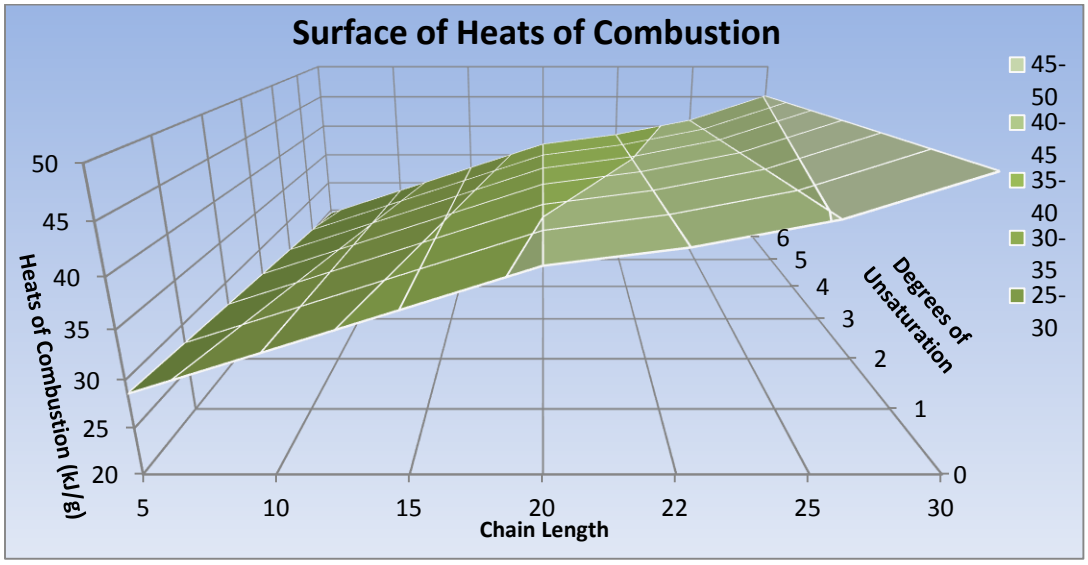
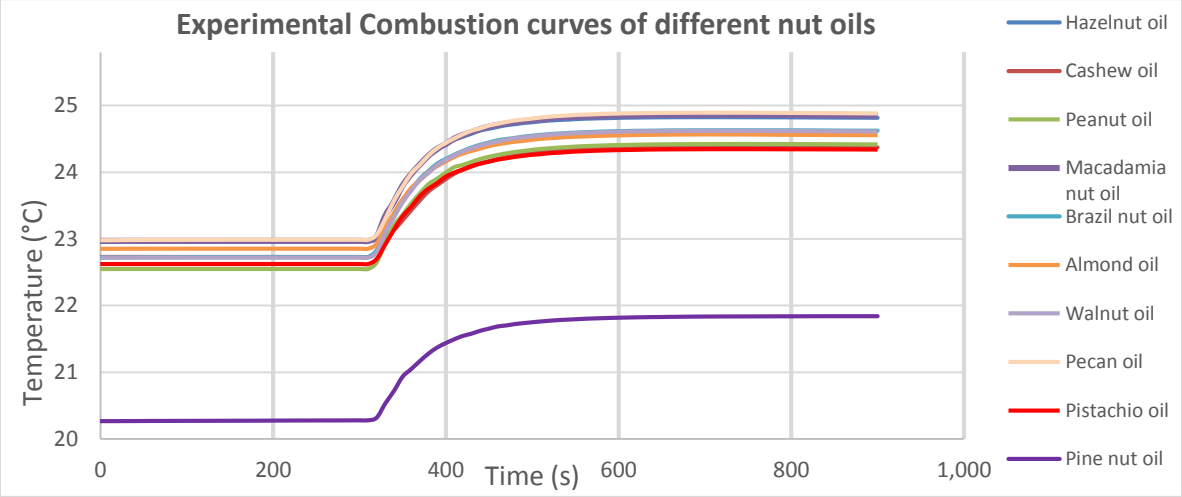


Table 3: Measured heats of combustions for nut oils

Nut oil	Average Heat of Combustion (kJ/g)
Pecan	39.115
Brazil nut	38.989
Macadamia	38.831
Hazelnut	38.445
Peanut	38.073
Walnut	37.830
Pistachio	35.187
Almond	34.601
Cashew	33.774
Pine nut	31.173

Figure 5: Bomb calorimetric data showing the time profile of temperature increase for nut oil runs



Calculations:

Calibration of heat capacity of calorimeter:

Heat capacity of the calorimeter is calculated according to the following formula:

$$(C_{cal} + C_{s,H2O}\, m_{H2O}) = (q_{s,BA}\, m_{BA} + q_{s,Fe}\, m_{Fe})/\, \Delta t \qquad (1)$$

- where $q_{s,BA}$, $q_{s,Fe}$, and $q_{s,H2O}$ are the known specific heats of combustion for benzoic acid (26.41 kJ/g) , iron (4.493×10^{-4} kJ/g), and water (4.184×10^{-3} kJ/g), respectively, m is the mass of the substance, C_{cal} is the heat capacity of the calorimeter without water, and Δt is the temperature change.

Average heat capacity of calorimeter including water (trials 1-3) is calculated as **10.15kJ/°C**.

The heat of combustion values for the standards and the nut oils are calculated using the average heat capacity of the calorimeter in Equation (1).

5. Discussion/Conclusion

Fresh, raw tree nuts were used for extraction of nut oils in order to obtain the heat of combustion of these nuts. The comparisons of these heats of combustion were based on the heats of combustion of nine pure standards and extracted DHA that were also combusted experimentally. The standardized heats of combustion contained sufficient distinction to allow for comparisons of fatty acid chain lengths and degrees of unsaturation in the tree nut oils. Excel's regression data analysis tool was used to formulate an equation for a 2D surface of heats of combustion as a function of chain length and degrees of unsaturation. The plot of this surface provides a practical way to determine the average chain length and average degree of unsaturation for any nut oil using its heat of combustion. Based on the results, the ranking of highest degree of unsaturation to lowest degree of unsaturation was found to be: hazelnut > pecan > Brazil nut > pistachio > peanut > cashew > macadamia. (The almond, walnut, and pine nut results were considered indefinite at this time due to the potassium oxide residue being present after combustion.) The ranking order of nuts in unsaturation agrees well in general with the USDA ranking. Both sets of data agree that hazelnuts have the highest percentage of unsaturated fats, followed second by pecans (Table 4). According to Table 4, a gradual decrease in degree of unsaturation is seen from hazelnut to macadamia when disregarding cashew and Brazil nut. There is only a slight difference between the values for macadamia and cashew which could be related to possible human error. Brazil nut, on the other hand, shows a much higher degree of unsaturation compared to the USDA ranking which requires further investigation into the source of discrepancy. This project demonstrated that it is possible to employ bomb calorimetry for the comparative study of the relative degrees of unsaturation of fatty acids in nuts. The major implication of this study is that other small college science departments lacking resources for costly instrumentation like McKendree University can employ this technique for a comparative study of lipids in different nuts as an undergraduate lab project.

Table 4: Ranking of nut oil compared to USDA

Nut	Ros and Mataix (USDA)	My Experimental Data	
	Fraction of UFA	Number of double bonds per carbon	Ranking
hazelnut	0.882	0.255	1
pecan	0.867	0.209	2
pistachio	0.829	0.167	4
peanut	0.813	0.135	5
macadamia	0.797	0.105	7
cashew	0.756	0.119	6
brazil nut	0.679	0.170	3

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