Extraction and $^1$H NMR Analysis of Fats from Convenience Foods: A Laboratory Experiment for Organic Chemistry

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Supporting Information

ABSTRACT: The extraction and analysis of fats from convenience foods (crackers, cookies, chips, candies) has been developed as an experiment for a second-year undergraduate organic chemistry laboratory course. Students gravimetrically determine the fat content per serving and then perform a $^1$H NMR analysis of the recovered fat to determine the levels of saturated, unsaturated, monounsaturated, and polyunsaturated fats in the food. Students compare their results to the information provided on the food package label. This experiment engages students in the learning process by connecting with their prior knowledge and familiarity of the foods used. Students learn several important concepts and techniques, such as fat characteristics and structure, solid–liquid extraction, structure–solubility relationships, vacuum filtration, and $^1$H NMR spectroscopy.

KEYWORDS: Second-Year Undergraduate, Hands-On Learning/Manipulatives, Laboratory Instruction, Organic Chemistry, Consumer Chemistry, Fatty Acids, Food Science, Lipids, Nutrition, NMR Spectroscopy

The incorporation of foods into the instructional laboratory is an often-used strategy to foster student interest and introduce relevance of scientific concepts to their everyday lives. A laboratory experiment is presented for a second-year undergraduate organic chemistry laboratory course in which students isolate fat from a “convenience” food and analyze the recovered fat using $^1$H NMR spectroscopy.

Structurally, fats are triglycerides (triacylglycerols) in which a glycerol backbone has been esterified with three fatty acids. A fatty acid is considered saturated if the carbon backbone possesses no alkenes and unsaturated if at least one alkene is present. Unsaturated fatty acids with only one alkene are considered monounsaturated, and those with more than one are polyunsaturated (Figure 1). The fats found in foods are not composed of one single structure but are complex mixtures of triglycerides.

In this experiment, students use solid–liquid extraction to isolate the fat content from a homogenized sample of a “convenience” food (crackers, cookies, chips, candies). The recovered fat is weighed and the result used to determine the mass of fat per serving in the food. Students then analyze the fat using $^1$H NMR spectroscopy. Although analyses of fats and fatty acids using IR spectroscopy, GC, and HPLC are well known, the use of NMR spectroscopy is less common. Due to the structural complexity of fats found in foods, chromatographic methods for analyzing fats usually require the transesterification of the triglycerides into simple esters prior to analysis. The use of $^1$H NMR spectroscopy simplifies the analysis of fats by avoiding this process and provides a view of the overall profile of the fats in the sample. Using $^1$H NMR spectral analysis, students are able to determine the mass quantities of saturated and unsaturated fat in the sample and to approximate the quantities of mono- and polyunsaturated fat. These values can then be compared directly to the information provided on the food package label.

By performing the experiment, students learn about fats, fat characteristics and structure, solid–liquid extraction, structure–solubility relationships, vacuum filtration, and $^1$H NMR spectroscopy. In addition, the experiment uses NMR spectral analysis as a tool for quantitative analysis, an important application not commonly encountered in undergraduate laboratory experiments.

EXPERIMENTAL OVERVIEW

Students working in pairs select a sample of a convenience food from a variety of provided options. The sample is then crushed and homogenized with a mortar and pestle. A small quantity (typically 1.0 g) of the crushed sample is placed in a centrifuge tube. The tube is sealed and the fat extracted by shaking the...
assignments. Students integrated the resonances for HA
Experimental Handout to assist students in making peak
of a representative fat. An example spectrum is included in the
by a student and the assignment of resonances to the protons
typical NMR spectrum of the fat extracted from an Oreo cookie
reported value.

should be evaporated under a hood. Chloroform-
results that ranged from 55
−
68% (76% average) of the
Students performed the extraction in an average of 1 h, with
saturated fats can be approximated by

Applying these percentages to the mass of total fat per
serving from the packaging provided the mass of saturated and
unsaturated fat that was compared to the package label.

Additionally, the relative quantities of mono- and poly-
unsaturated fats can be approximated by

Percent Polyunsaturated Fat = \left( \frac{H_f - \frac{2H_c}{3}}{H_c} \right) \times 100%

Again, each fatty acid residue must have two \( \alpha \)-hydrogens
\( (H_\alpha) \), so half of this integral value represents a “total” number
of fatty acids. Most polyunsaturated fatty acids are “methylene
interrupted” dienes or trienes. Thus, diunsaturated fatty acids
have two bis-allylic hydrogens \( (H_b) \) and triunsaturated fatty
acids have four. The most common triunsaturated fatty acid is
\( \alpha \)-linolenic acid (the bottom fatty acid residue on the fat
depicted in Figure 2). An \( \omega-3 \) fatty acid, \( \alpha \)-linolenic acid has
methyl protons \( (H_m) \) that are homoallylic, and are thus seen
slightly downfield compared to the methyl protons \( (H_m) \) of
other fatty acids. Thus, the number of triunsaturated fatty acids
can be approximated as one-third of the integral value of \( H_b \).
Subtracting twice this value from the bis-allylic hydrogens \( (H_b) \)
removes the “extra” hydrogens contributed by the triunsatu-
stated fatty acids. Half of this difference represents the
number of polyunsaturated fatty acid residues present in the fat.
The remaining fat represents the percentage of monounsatu-
rated fat in the sample according to

Percent Monounsaturated Fat = \frac{\text{Percent Unsaturated Fat} - \text{Percent Polyunsaturated Fat}}{2}

Applying these percentages to the mass of total fat per
serving from the packaging provided the mass of mono- and
polyunsaturated fat that was compared to the package label.

tube vigorously for at least 1 min with 2–3 mL of hexanes.
Undissolved solids are removed via vacuum filtration through a
small pad of Celite and washed with an additional 2–3 mL of
hexanes. The filtrate is dried over anhydrous sodium sulfate for
approximately 5 min to remove any water introduced from the
food sample and decanted into a small preweighed beaker.
Solvent is removed by evaporation with gentle heating from a
hot plate under ventilation from a fume hood or snorkel. The
beaker is then reweighed to determine the mass of the extracted
fat. A small quantity (5−10 mg) of the fat is dissolved in
chloroform-d and the solution transferred to an NMR tube for
\(^1\)H NMR analysis.

■ HAZARDS

Hexanes are flammable and contain \( \eta \)-hexane, a neurotoxin, and
should be evaporated under a hood. Chloroform-d is a
suspected carcinogen.

■ RESULTS AND DISCUSSION

Extraction of Fat

Five pairs of students performed the extraction using Oreo
Cookies and four pairs of students used Goldfish Crackers.
After evaporation of hexanes, the mass of recovered fat from
the 1 g food sample was determined (typically around 100−200
mg) and the result used to calculate the quantity of total fat that
would be recovered in a full serving. This value was compared
to the total fat per serving reported on the food packaging label.

Students performed the extraction in an average of 1 h, with
results that ranged from 55−88% (76% average) of the
reported value.

\(^1\)H NMR Analysis

The analysis of the recovered fat using \(^1\)H NMR spectroscopy
showed the samples were quite pure. Peaks were sufficiently
resolved at 300 MHz to perform the analysis. Figure 2 shows a
typical NMR spectrum of the fat extracted from an Oreo cookie
by a student and the assignment of resonances to the protons
of a representative fat. An example spectrum is included in the
Experimental Handout to assist students in making peak
assignments. Students integrated the resonances for H_\alpha, H_\beta, H_\gamma, and H_\delta to determine the quantities of saturated and unsaturated
fat in the sample and to approximate the quantities of mono-
and polyunsaturated fat.

Although the quantity of unsaturated (and, therefore,
saturated) fat can be calculated from the NMR data several
ways, the best results were found using the integrals of the \( \alpha \)
hydrogens \( (H_\alpha) \) and allylic hydrogens \( (H_\beta) \) according to

Percent Unsaturated Fat = \frac{H_\beta}{2H_\alpha} \times 100%

Because each fatty acid residue must have two \( \alpha \)-hydrogens
\( (H_\alpha) \), half of this integral value represents a “total” number
of fatty acids. Because each unsaturated fatty acid residue must
have four allylic hydrogens \( (H_\beta) \), one-quarter of this integral
value represents the number of unsaturated fatty acid residues.
The remaining fat represents the percentage of saturated fat in
the sample according to

Percent Saturated Fat = 100% − Percent Unsaturated Fat

Applying these percentages to the mass of total fat per
serving from the packaging provided the mass of saturated and
unsaturated fat that was compared to the package label.

Percent Polyunsaturated Fat = \left( \frac{H_f - \frac{2H_c}{3}}{H_c} \right) \times 100%

Percent Monounsaturated Fat = \frac{\text{Percent Unsaturated Fat} - \text{Percent Polyunsaturated Fat}}{2}

Applying these percentages to the mass of total fat per
serving from the packaging provided the mass of mono- and
polyunsaturated fat that was compared to the package label.

Figure 2. Example \(^1\)H NMR spectrum of fat recovered from the extraction of an Oreo referenced to a “hypothetical” fat.
A total of approximately 60 students have performed this experiment in the last two years in the second semester of an introductory organic chemistry course. Tables 1 and 2 show the results from three classes for 18 students. At least in the United States, fat content on food labels is typically reported to the nearest 0.5 g. The values calculated from the $^1$H NMR data were typically quite close to the values provided on the food packaging label.

**CONCLUSION**

Students successfully isolated the fat from "convenience" foods, determined the fat content per serving, and calculated the mass of saturated, unsaturated, monounsaturated, and polyunsaturated fats in the food using $^1$H NMR spectral analysis. The experiment took about an hour to perform, and an additional 10 min was needed for the students to acquire an NMR spectrum and integrate the necessary peaks. Students who participated clearly enjoyed the experiment and learned about fats and their structural characteristics, solid–liquid extraction, structure–solubility relationships, vacuum filtration, and NMR spectroscopy and its use as a tool for quantitative analysis.

**ASSOCIATED CONTENT**

3 Supporting Information

Experimental handouts for students; notes for instructors including results for a wide variety of food samples; a detailed explanation of the derivation of eqs 1–4. This material is available via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**


6 For recent examples of laboratory experiments using NMR as a quantitative tool, see (a) Cook, A. G.; Felman, P. M. Determination of Solvent Effects on Keto–Enol Equilibria of 1,3-Dicarbonyl Compounds Using NMR. *J. Chem. Educ.* 2007, 84 (11), 1827–

(7) A field strength of at least 200 MHz is recommended to resolve the H₁ and H₂ peaks adequately. The H₂ peak was not observed in many of the foods tested (especially candies), which may allow the use of lower field strength instruments in those instances.

(8) A detailed explanation of the derivation of eqs 1−4 is available in the Supporting Information.