Second-Year and AP Chemistry

Classroom Research: GC Studies of Linoleic and Linolenic Fatty Acids Found in French Fries

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There are many advantages to giving students an opportunity to conduct research before they graduate from high school or college. This type of experience can be very rewarding for students as well as for teachers and institutions. The most notable advantage is that students engage in critical thinking and their own analysis of a problem they have identified.

Designing a chemistry research class for seniors in high school or undergraduates in college presents many challenges. With the cooperation of the administration and proper selection of students, this successful classroom research model may be utilized elsewhere.

Course Logistics

The students met daily for 45 minutes for two semesters. Each student had a task, such as using the Internet to investigate the link between cancer and fatty acids, searching the *Journal of Chemical Education* Web site to learn the requirements for submitting articles, obtaining French fries from different fast food restaurants, beginning a methyl ester synthesis, or injecting the reference sample or fry sample into the gas chromatograph (GC). Primarily, the students spent time thinking critically and analyzing the results of the GC injections.

The group comprised eight senior girls, four senior boys, and one junior girl. The two-semester class, Advanced Chemical Research, was open to students who had completed Advanced Placement Chemistry and received recommendation from the instructor. The students were required to submit an essay explaining why they wanted to take the research class and what they hoped to contribute and accomplish. This assignment acted as a brainstorm instrument to help them clearly establish their desired goals.

Student Selection

The following course description was distributed to the students.

> The research class requires using the Internet for researching journal articles from a wide variety of scientific and medical related publications. Persons interested should be self-directed learners, intellectually motivated in science, possess intuitive skills, and demonstrate their ability to be a team player. Students must be flexible since the research may require meeting outside of the regularly scheduled class period. Students will learn how to prepare samples and to inject them properly into the GC. Students will keep an organized notebook of journal articles and text materials related to their project. Proper scientific documentation in daily science lab books will be kept by the students and graded by the supervising teacher. Students will be required to meet once a week in a round table, student led discussion, to reveal their findings and suggest future experimentation. Students interested in taking this research class must have AP Chemistry as a prerequisite. They will also prepare and present their research to high school and college audiences. Students are required to write a letter of why they believe they should be selected to participate in this class.

Selecting an Interesting Research Topic

Research dealing with the link between diet and cancer was of great interest to the students. Students read research articles from The Association for the Cure of Cancer of the Prostate (CaP CURE), a cancer research program funded by the Milken Family Foundation, which yielded interesting data. The instructor of the research class contacted CaP CURE to find a suggested protocol for preparing food samples for gas chromatograph injections (*1*). Samples of tumors removed from women with breast cancer had high ratios of linoleic to linolenic acid. Epidemiologic studies of women with breast cancer suggested a link between the rate of growth of breast tumors and the ratio of these acids. When the ratio of linoleic to linolenic acid was approximately 9 to 1, growth was accelerated (*2, 3*).

Since linoleic and linolenic acids are found in cooking oils (*4*), it was decided that an investigation of the ratios of these two fatty acids in French fries should be conducted. The students examined publications regarding nutrition and health (*5*). They decided to try to separate linoleic and linolenic acids by using a gas chromatograph or high-pressure liquid chromatography. A gas chromatograph was selected for this classroom setting because of its ease of use and accuracy, and the length of time needed to complete a run.

Equipment and Materials

Wichita Collegiate leases an Agilent 6850 gas chromatograph. The 6850 GC, a smaller, less expensive version of the HP 6890, fits into the 10 × 12-foot room that is used for research and lab preparation. Premium-grade gases are used: helium for carrier gas, hydrogen and air for FID, and nitrogen to create inert conditions during synthesis. A DB-23 capillary column works best for separating methyl linoleate and methyl linolenate. A centrifuge and an analytical balance are needed for the synthesis. Sample 16A (GLC reference mixture) from Nuchek-Prep Inc.[1] had the following methyl

esters in percent by weight: methyl palmitate, 7.0%; methyl stearate, 5.0%; methyl oleate, 18.0%; methyl linoleate, 36.0%; methyl linolenate, 34.0%.

Synthesis Procedure

To separate linoleic and linolenic acids, it is first necessary to convert them to nonpolar derivatives. The general reaction $RCOOH + CH_3OH \rightarrow HOH + RCOOCH_3$ is carried out with an acidic catalyst. A textbook procedure (*6*) was followed with a few modifications. Students encountered difficulty with the amount of water retained in the sample in this procedure. William Groutas, an organic chemist from Wichita State University, assisted them by suggesting an acidic anhydrous catalyst. The following successful procedure was used to change the fatty acids in French fries to methyl esters.

The students weighed four 8.0-mL vials with caps. A one-centimeter piece was cut from the middle of a French fry with a razor. The sample was placed in vial 1 and sliced finely with a 5-mm flat-head Exacto knife. Under a fume hood, 3.0 mL of chloroform and 1.0 mL of anhydrous methanol were added to vial 1. The vial was shaken for five minutes to extract the fatty acids, and then the solid particles were allowed to settle. The liquid was transferred to vial 2, using a glass pipet with cotton wrapped around the tip to separate the solution from solid French fry particles. The separation process was repeated two more times, adding chloroform and methanol to vial 1 and transferring to vials 2 and 3.

A 5.0-g sample of sodium sulfate was added to each of two centrifuge tubes. The contents of vials 2 and 3 were equally distributed between the two tubes. The tubes were centrifuged for 30 minutes. The same separation method was used to add the liquid contents from the centrifuge tubes to vial 4. The solvent from vial 4 was evaporated using a stream of nitrogen. Then vial 4 was placed in an ice bath and 5.0 mL of anhydrous methanol and 0.50 mL of acetyl chloride were added. The vial was capped and left standing overnight at room temperature. Again, the solvent was evaporated under a stream of nitrogen. The weight of the oil and vial 4 was recorded, then the sample was diluted with dichloromethane (*6*). Using a splitless method, 1.5 mL of the methyl ester solution was directly injected into the gas chromatograph.

Instructor's Notes on Injection

An Agilent 6850 GC was used to analyze the samples. The column, DB-23, was 30 m long with a 0.25-mm inside diameter. One microliter of Nu-Chek sample 16A was injected. The initial column pressure was 12.00 psi; this was held for 6.50 min, then reduced to 8.00 psi at 2.00 psi/min and held for 12.00 min. The intial oven temperature was 170 °C; this was programmed to increase to 230 °C at 6.00 °C/min, and held constant at 230 °C for 2 min. The total run time was 12.00 min. The inlet parameters and FID parameters are shown in the box (*7*).

Inlet Parameters	Flame Ionization Detector Parameters
Splitless: carrier gas, helium	Heater, 300 °C
Heater, 300 °C	Hydrogen flow, 40.00 mL/min
Pressure, 12.00 psi	Air flow, 450 mL/min
Total flow, 32.8 mL/min	Helium flow, 8.8 mL/min

The ratio of methyl linoleate to methyl linolenate in the Nu-Chek 16A sample was 1.078 to 1.000. The GLC reference mixture company standard for 16A was 1.059 to 1.000. The runs have corresponding peaks at 9.34 and 10.32 minutes. One microliter of methyl-esterified solution from each sample French fry was injected and compared to the Nu-Chek standard. The methyl linoleate and methyl linolenate peaks in the fry showed up at 9.34 and 10.32 minutes.

Hazards

The principal chemical hazards are acetone, dichloromethane, acetyl chloride, methanol, and chloroform. When handling any of these chemicals it is always necessary to use proper protection, such as lab goggles and gloves, because they are irritants to the skin, eyes, and respiratory tract. These chemicals are hazardous if ingested or inhaled; therefore, all experiments must be conducted under a fume hood. Chloroform and dichloromethane are cancer-suspect agents.

Analysis

Students analyzed the chromatograms of French fry samples from five restaurants. This was where they applied their critical thinking. After finding the retention times of linoleic and linolenic acids in Nu-Chek sample 16A from the chromatogram in Figure 1, they used this chromatogram as a reference point to identify the peaks of methyl linoleate and methyl linolenate in their French fry samples. They then compared the area under the peak of methyl linoleate to the area under the peak of methyl linolenate for the samples from the restaurants.

Table 1 shows the calculated values of the integration under the peaks in Figure 2 (chromatogram for restaurant A). French fries from restaurant A had an average ratio of methyl linoleate to methyl linolenate of 17.19 to 1.00 with a standard deviation of ±0.91. This is considerably greater than the 9 to 1 ratio at which tumor growth is accelerated. Four of the five restaurants yielded ratios higher than 9 to 1.

The sample from restaurant B, however, had an average ratio of methyl linoleate to methyl linolenate of only 3.250 to 1 with a standard deviation of ±0.11, far less than 9 to 1, as shown in Figure 3. The chemistry teacher spoke with the owner of restaurant B to discuss the process used to cook the French fries. The oil was a corn–vegetable combination typically used in other restaurants. However, these French fries are coated before cooking with a substance unknown to the students. Until further testing, the research group has tentatively concluded that the lower ratio is due to the outside variable (coating), since the cooking oils used in all five restaurants are essentially the same. Only one restaurant double coats its fries: the one that had lower linoleic-to-linolenic ratios.

After looking at their analysis, the students decided to test homemade fries cooked in different oils to check the ratio of fatty acids absorbed. In addition to testing different oils, they planned to use a stationary basket and a rotating basket while frying to see if there is a difference in ratio of the fatty acid absorption as claimed by some companies selling certain types of fryers. The other variable they planned to test was the different coatings used on fries. This required research on what coatings are currently being used. Many Web sites of restaurants reveal a partial list of items in their fries.

Table 1. Values Calculated from Chromatogram for Restaurant A

Ratio, Linoleic/ Linolenic Acid	Linoleic Acid, Linolenic Acid			Synthesis Date
	Peak No.	Retention Time/min	Area/pA	
17.72	15, 17	9.137, 10.08	858.3, 48.45	4/03/2001
19.01	15, 17	9.141, 10.07	872.6, 45.90	4/03/2001
17.39	14, 16	9.133, 10.08	755.4, 43.43	4/03/2001
17.08	8, 11	9.188, 10.09	1630, 95.45	3/05/2001
16.59	8, 11	9.142, 10.08	981.6, 59.14	3/05/2001
15.76	8, 10	9.133, 10.06	1232, 78.14	3/05/2001
18.10	8, 9	9.115, 10.06	774.9, 42.80	3/05/2001
17.52	8, 10	9.139, 10.07	1076, 61.44	3/05/2001
16.92	8, 10	9.111, 10.05	831.9, 49.18	2/08/2001
16.77	10, 11	9.076, 10.04	525.3, 31.32	2/08/2001

Grading Rubric

Students were graded weekly according to the following categories: daily assignments, individual effort, teamwork, cleanup, synthesis technique, injection technique, computer work, attendance, and PowerPoint and poster presentations. Each category was assessed on a 50-point scale. This rubric provided a fair assessment of each student and the role he or she played in the research class. Every nine weeks, the students turned in a self-evaluation using the rubric provided by the teacher, and the teacher also graded each student. A comparison of the student self-evaluation and the teacher evaluation was an excellent opportunity for discussion of individual growth whenever there was a large disparity between the two evaluations.

Presentation of Results

The students presented their research to Friends University, Wichita State University (WSU), the Wichita Collegiate Board of Trustees, scientists from Koch Industries, KU-School of Medicine, and Olathe North High School. At Friends, the audience comprised American Chemical Society students, seniors majoring in chemistry, and two professors. The interaction was extremely beneficial for the research students and the audience from Friends University. The Collegiate students gained confidence from sharing their research, and the senior chemistry students from Friends felt a definite need to upgrade the gas chromatograph that they were using for research.

Five professors from WSU listened to the research presentation. These professors had different chemistry backgrounds: organic, inorganic, and medicinal chemistry and biochemistry. Their feedback gave the students ideas for future studies. The professors complimented the students on their work and supported the instructor by emphasizing the importance of reproducible results, changing one variable at a time, and using standards for comparison.

The scientists from Koch Industries affirmed the precision of the students' GC injection technique. They gave the students a tour of their research and development department, demonstrating real-life applications of GC. The students learned that many aspects of the oil industry do not require special sample synthesis preparation before injection. Most oil samples can be directly injected into the GC. The students discovered that GC research on oils is much simpler than GC research on French fries.

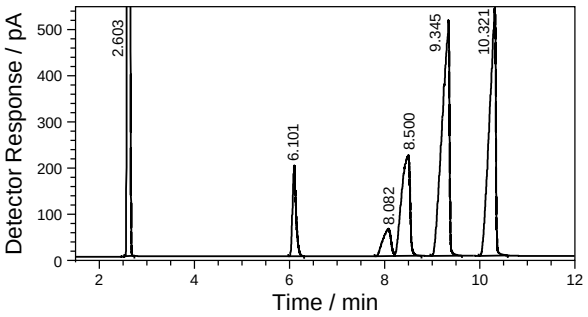

Figure 1. Chromatogram for Nu-Chek sample 16A.

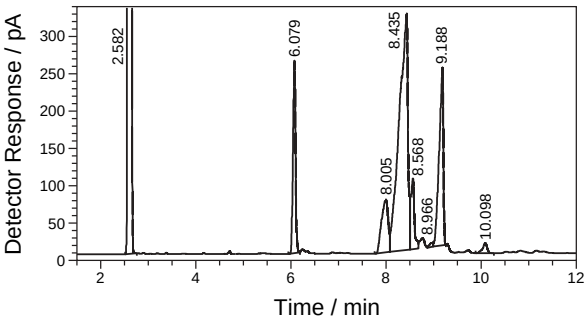

Figure 2. Chromatogram for French fry sample, restaurant A.

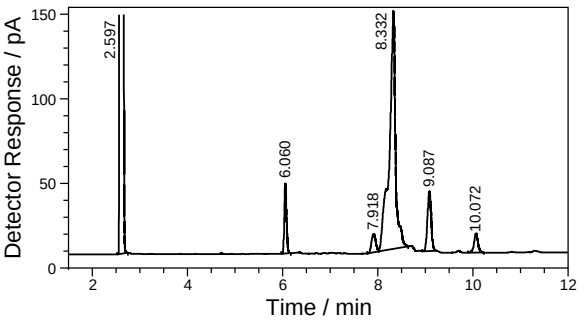

Figure 3. Chromatogram for French fry sample, restaurant B.

The Olathe North High School visit included students from AP Biology and AP Chemistry and their teachers. The teachers and students are planning a proposal to acquire a GC for their school to do similar research projects. Eventually the Collegiate and Olathe North would like to work on joint research projects.

The students also had an opportunity to do a poster presentation at the University of Kansas School of Medicine Tenth Annual Research Forum. They were the only high

school students invited to present along with medical students, professors, and physicians.

Discussion

Next year, the new research group from Wichita Collegiate plans to investigate the coating used on French fries that prevents complete absorption of linoleic and linolenic acid. The group will begin where this year's group ended.

There appears to be a new surge of high schools and colleges interested in doing entry-level research with students (*8*). The gas chromatograph is one of the least expensive instruments that will hold up over time with little maintenance. It offers a fast, easy method for students interested in pursuing a research topic. When students take chemistry and AP Chemistry they learn excellent chemistry, but they do not experience the essence of science as an investigative endeavor. Research permits them to use their intellect and critical thinking skills to solve a problem that applies to their lives. Also, the structure of this research class allows the teacher to meet the demands of teaching other courses concurrently. The greatest gain for students is the opportunity to put their intuitive skills to work on a daily basis.

It is recommended that the instructor team with a local scientific company or university to assist the project. Suzanne Henning with CaP CURE, a Milken Family Foundation Cancer Research Group, provided immense support for this group by suggesting a study of foods cooked in oils high in linoleic acid. William Groutas, from the Chemistry Department at WSU, provided excellent organic synthesis tips that removed water and improved the quality of injection sample.

Our school leaders are pleased with the research class. The board has made a commitment to continue offering it. Former research students say they are utilizing the knowledge gained from this unique research experience in their college work. For example, Megan Marshall, who is attending Duke University, discussed the research Wendy Demark-Wahnefried was doing at Duke Hospital. Her research has to do with the effect of low-fat diet on prostate cancer. She was specifically looking at the inhibition effects of linolenic acid. This may lead to a summer internship for Megan.

Note

1. Nu-Chek, P.O. Box 295, Elysian, MN 56028. Distributor of prepared chromatographically pure, lipid, organic, compounds.

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