Hypothesis

Sunflower oil is a polyunsaturated oil compressed from sunflower (*Helianthus annnuus*) seeds. Like most polyunsaturated fats, it is most commonly a triglyceride composed of glycerol and three unsaturated fatty acid chains. An example of a sunflower oil triglyceride is shown below. (Thomas, 2002)

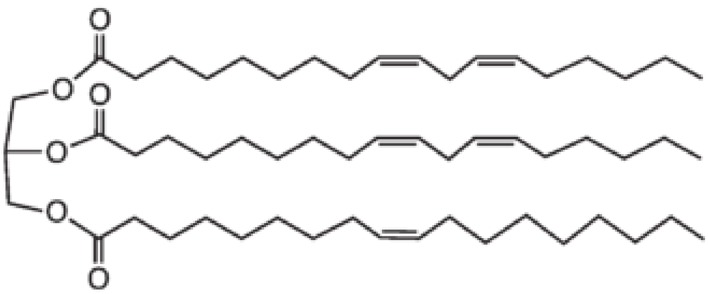

Figure 2: A sample sunflower oil triglyceride with 2 linoleic acid (doubly unsaturated) fatty acid chains and one oleic (monounsaturated) fatty acid chain.

(Smokefoot, 2011)

The term "unsaturated" refers to the presence of one or more carbon-carbon double bonds in the fatty acid hydrocarbon chain. Triglycerides with these unsaturated fatty acids undergo oxidative rancidity, a process which follows a free radical reaction mechanism.

Free radicals are formed by homolytic fission. In the context of fatty acids, the C-H bond in the fatty acid chain undergoes homolytic fission to form two free radicals:

RH → R· + H·. This step is called initiation.[2]

Next, the radicals undergo a free radical chain reaction, in which O_2 from the air is added to the fatty acid free radical (R•) to form ROO•. ROO• then reacts with a fatty acid molecule to form R• and a hydroperoxide (ROOH).

R· + O2 → ROO·

ROO· + RH → ROOH + R·

In the final step, termination, the ROOH hydroperoxide decomposes into RO• + OH•. These, along with the other free radicals present, react to form non-radical products, including aldehydes, ketones, alcohols, esters, and alkanes. The products are collectively referred to as hydroperoxides. (Derry, 2009)

EX: This is not the termination step.

C: These are not called hydroperoxides.

[2] The symbol "•" is used to denote a free radical. "R" represents the part of the hydrocarbon chain attached to the H

The first step of this reaction, initiation, requires energy to begin. Because of this, heat can act as a catalyst for the reaction. (Derry, 2009)

In order to test for the amount of oxidation in a sample of sunflower oil, the amount of hydroperoxides produced can be found. Hydroperoxides, particularly alcohols and aldehydes, can be oxidized by acidified potassium dichromate (IV). When oxidation occurs in this reaction, the orange solution containing dichromate (IV) ions is reduced to a green solution containing chromium (III) ions. The electron half-equation for this reaction is as follows:

C: Continued wrong use of nomenclature.

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$$

(orange) (green)

The more sunflower oil is oxidized, the more hydroperoxides are produced. (Clark, 2003) Consequently, the more hydroperoxides are produced, the more green light is emitted and red light (its complementary color) is absorbed by the potassium dichromate (IV) solution. This is expressed in the form of:

$$A = ebc$$

in Beer's Law, where A is absorbance, e is the molar absorbtivity ($Lmol^{-1}cm^{-1}$), b is the path length of the sample, and c is the concentration of the compound in the solution ($molL^{-1}$). Beers law indicates a directly proportional relationship between absorbance and concentration of absorbing compounds. Thus, the absorbance of red (630 - 700 nm) light by a solution of potassium dichromate (IV) and oxidized sunflower oil is directly proportional to the concentration of hydroperoxides in the solution. (SHU, n.d.)

Given this relationship, colorimetry can be used to determine the relative concentration of hydroperoxides produced in sunflower oil at different temperatures. As stated above, heat can act as a catalyst for the reaction as initiation requires energy to begin. Therefore, as storage temperature increases, so should the concentration of hydroperoxides, and consequently the absorbance of red light over a controlled period of time.

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Procedure

1. Plug in and turn on the colorimeter. Allow 0.5 hr for the machine to warm up.
2. Using a 10.0 mL glass pipette and pipette filler, fill 1 test tube with 8.5 mL of 1M potassium dichromate (IV).
3. Using a 5.0 mL glass pipette and pipette filler, add 1.0 mL of .01M sulfuric acid to the test tube to acidify the solution.
   Acidifies the solution and provides the H^+ ions necessary to catalyze the oxidation of hydroperoxides in the sunflower oil.

EX: A stock solution of greater volume should be prepared.

4. Rinse the pipette. Then, using a new 5.0 mL glass pipette, add 0.5 mL sunflower oil to the test tube.
   A new pipette is used to avoid cross-contamination.
5. Move test tube in a figure eight motion to allow the reactants to mix.
6. Using a 10.0 mL glass pipette and pipette filler, fill a cuvette with 8.0 mL of the mixed solution.
7. Zero the colorimeter.
   Controls the base of measurement for the colorimeter.
8. Select a wavelength of 635 nm.
   Controls the wavelength of light absorbed by the solution.

C: 635 or 670 nm? Not clear.

9. Polish the cuvette and insert it into the chamber of the colorimeter. Record the absorbance of 670 nm light. This is the baseline absorbance of sunflower oil.
   Cuvette is polished in order to minimize the interference of dirt on the absorption of light by the sample. Baseline is recorded to control the relative measurement of the later samples.

Confusion over wavelength again..

10. Using a thermometer (°C), determine and record the temperature of the refrigerator, the freezer, and the room.
11. Using a 100.0 mL measuring cylinder, measure out 50.0 mL of sunflower oil. Pour into a 100.0 mL glass beaker.
    Controls the volume (mL) of sunflower oil, and the volume (mL) of the beakers.
12. Repeat step eleven for all fifteen beakers.
    Ensures that all fifteen beakers are under the same conditions.
13. Wrap all beakers in aluminum foil.
    Controls the exposure to light of the sunflower oil. Because light exposure can also affect or catalyze the reaction, this control is crucial to isolating just one independent variable.
14. Prepare a 40 °C water bath and a 60 °C water bath.

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15. Place 3 foil-wrapped beakers into each water bath, and 3 foil-wrapped beakers each into a 4 °C refrigerator, a -20 °C freezer, and a box at room temperature.

Sets five different values for the independent variables and three different trials per value in order to decrease the likelihood of random error.

16. Over the course of the next 72 hours, refill the water baths so they do not evaporate.

If a water bath dries up, it could pose a potential fire hazard.

17. After 71.5 hours, plug in and turn on colorimeter so as to allow .5 hr for warmup.

18. Using a 10 mL glass pipette and pipette filler, fill 15 test tubes with 8.5 mL of 0.1M potassium dichromate (IV).

Controls the volume of 1M potassium dichromate (IV) in the test tubes.

19. Using a 5 mL glass pipette and pipette filler, add 1 mL of 2 M sulfuric acid to each test tube to acidify the solution.

EX: Should make up a stock of this solution.

Controls the volume of 2 M sulfuric acid in the test tubes.

20. Rinse the pipette. Then, using the same 5.0 mL glass pipette, add 0.5 mL of oxidized sunflower oil from each beaker into its corresponding test tube. Be sure to rinse the pipette thoroughly between samples to avoid cross-contamination.

EX: Not the best control of volumes.

EX: How long is the mixture allowed to react before measuring absorbance?

Rinsing controls the purity of the samples.

21. Place all test tubes on test tube rack. Move test tube rack in a figure eight motion to allow for mixing of reactants.

EX: Not an ideal method of mixing.

Moving the entire rack together controls the path of movement of the test tubes, thus allowing the movement of the reactants within the tubes to be controlled to an extent.

22. Using a 10.0 mL glass pipette and pipette filler, fill each of 15.0 cuvettes with 8.0 mL of the corresponding mixed solution.

Controls the volume (mL) of oxidized hydroperoxide solution in each cuvette.

23. Zero the colorimeter.

Controls the base of measurement for the colorimeter.

24. Select a wavelength of 635 nm.

Controls the wavelength of light absorbed by different samples.

25. Polish each cuvette and insert it into the chamber of the colorimeter. Record the absorption of 635 nm wavelength light.

Cuvette is polished in order to minimize the interference of dirt on the absorption of light by the sample.

26. Remove the cuvette from the machine for each sample. Clean up station, washing all materials and very carefully washing and storing colorimeter cuvettes away from other test tubes.

- For each of 3 trials of 5 different independent trial values, the absorbance (A) of 670 nm wavelength light will be recorded.
- To find the rate of reaction for each trial, the absorbance will be set over the total amount of time of the reaction.
- Given that Beer's Law states that absorbance is proportional to concentration, A/hr $\propto$ Concentration/hr, and so in terms of rate the units will be (hr^{-1}).
- The average rate of oxidation for each independent variable value will be calculated using the standard formula for mean: mean = (a + b+ c)/n, where n = the number of values being averaged, in this case 3.
- These average rates will then be plotted on a graph of Temperature (℃) vs. Rate of Oxidation (hr^{-1}), and the slope of the line of best fit will be found.

Or 635?

Safety Precautions

Students should be cautious with using water baths. If they dry out, they pose a potential fire safety hazard. It is thus important that they always have water in them. In addition, potassium dichromate is one of the most common causes of chromium dermatitis. (Masters, 2003) As with all Cr^{VI} compounds, potassium dichromate is carcinogenic, and thus must be handled with gloves and safety goggles, and worked with under a safety hood.

EX: Good consideration of safety issues.

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Data Collection and Processing

Qualitative Observations

The 15 beakers of sunflower seed oil appeared identical as they were placed into their respective storage temperatures. The oil was a light yellow color and quite transparent. After a period of 72 hours, some obvious changes in appearance occurred. For example, the oil stored at -20 °C was frozen solid and had to be thawed before experimented upon. The oil stored at 60 °C became cloudy and more translucent. When the potassium dichromate was acidified with 2M sulfuric acid, the reacting solution gave off heat, and the test tubes were hot to the touch. Finally, when the oil was added to acidified potassium dichromate, no obvious color change was observed.

Table 1: Base Measurements for the Absorbance (A) of Sunflower Seed Oil After it has Reacted With Potassium Dichromate (IV) (±.001)[3]

Solution	Absorbance (A) (±.001)
Distilled Water (H2O)	0.000
Unoxidized Sunflower Seed Oil Reacted with (concentration) Potassium Dichromate (IV) solution	-0.050

A: Is this the baseline used in the end?

Table 2: Absorbance (A) of Oxidized Sunflower Seed Oil After Storage at Different Temperatures and a Reaction with Potassium Dichromate (IV) (±.001)

Storage Temperature	-20.0	4.0	25.0	40.0	60.0
Absorbance (A) (± .001)					
Trial 1	0.020	0.037	0.039	0.068	0.060
Trial 2	0.030	0.033	0.043	0.061	0.095
Trial 3	0.022	0.035	0.041	0.059	0.078

A: No comment on the variance and possibility of outliers.

Table 3: Average (Mean) Absorbance of Oxidized Sunflower Seed Oil After Storage at Different Temperatures (°C) and a Reaction with Potassium Dichromate (IV)

Storage Temperature (°C)	Mean Absorbance (A)
-20.0	0.024 ± 5.00%
4.0	0.035 ± 3.03%
25.0	0.041 ± 2.56%
40.0	0.063 ± 1.69%
60.0	0.078 ± 1.67%

[3] Uncertainty of the colorimeter

Calculation of the mean absorbance (A):

Formula used: $A_{avg} = \sum A/n$, n = number of trials (3 in this case)

Sample calculation: mean absorbance for oil stored at -20.0℃:

$A_{avg} = \sum A/n$

= (0.020 + 0.030 + 0.022) / 3 = 0.024

Calculation of uncertainty:

In this instance, the highest percent uncertainty for each storage temperature was taken as the uncertainty of the average.

Formula used: $\%U = U_a / A \cdot 100$, where U_a is the absolute uncertainty and A is the absorbance for each datum collected.

Sample calculation: % uncertainty for oil stored at -20.0℃:

$\%U = U_a / A \cdot 100$

= 100 (**(.001/0.020)**, (.001/0.030), or (.001/0.022))

= .050 · 100 = 5.00%

Table 4: Relative Rate (hr^{-1}) of Oxidation of Sunflower Seed Oil at Different Storage Temperatures

Storage Temperature (°C)	Average relative rate of oxidation (hr^{-1})
-20.0	$3.33 \cdot 10^{-4} \pm 5.69\%$
4.0	$4.86 \cdot 10^{-4} \pm 3.72\%$
25.0	$5.69 \cdot 10^{-4} \pm 3.25\%$
40.0	$8.75 \cdot 10^{-4} \pm 2.38\%$
60.0	$1.08 \cdot 10^{-3} \pm 2.36\%$

C: Too many sig figs compared to input absorbance data.

Calculation of average relative rate of oxidation:

Formula used: rate = A_{avg}/time

Sample calculation: average relative rate of oxidation of oil stored at -20.0℃:

rate = A_{avg}/time

= 0.024 / 72 hr = $3.33 \cdot 10^{-4}$ hr^{-1}

A: Should the student be taking into account the unoxidized oil absorbance to calculate a rate of change?

A: The units should be $A.hr^{-1}$ in this case.

Calculation of uncertainty

Formula used: $\%U_{rate} = \%U_A + \%U_{time}$[4]

Sample calculation: %U of oil of the average relative rate of oxidation of oil stored at -20.0℃

$\%U_{rate} = \%U_A + \%U_{time}$

= 5.00% + .694% ≈ 5.69%

[4] Absolute uncertainty for time is assumed .5 hr. For %U, .5 / 72.0 * 100 = .694%

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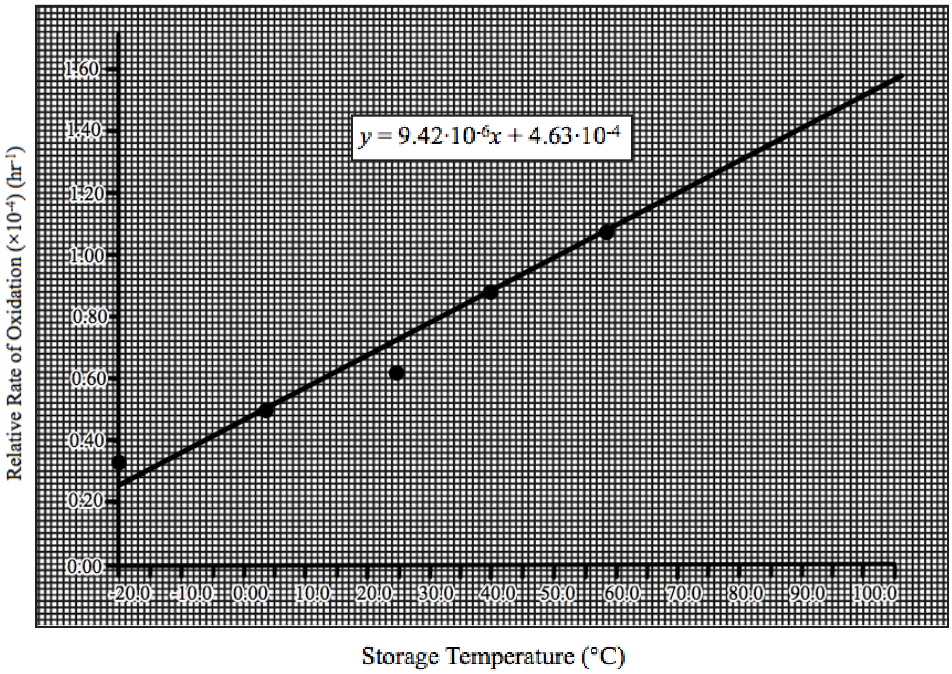
Figure 3: Graphical Representation of the Relative Rate of Oxidation (hr^{-1}) of Sunflower Seed Oil Versus its Storage Temperature (°C) Over a Period of 72 hr.

The equation of the line of best fit is $y = 9.42 \cdot 10^{-6}x + 4.63 \cdot 10^{-4}$. This is indicative of a positive correlation between storage temperature of sunflower seed oil and its rate of oxidation.



Conclusion and Evaluation

This laboratory experiment has determined that the hypothesis, which states that if the storage temperature (°C) of sunflower oil increases, then the rate (hr^{-1}) of oxidation will increase, is true.

Table 4 shows the mean relative rate of oxidation of sunflower seed oil. As predicted in the hypothesis, the storage temperature and rate of oxidation of sunflower seed oil display a directly proportional relationship. This correlation is supported by the graph in Figure 4, which clearly shows an upward trend. It should be noted that the slope of the line determined from this experiment's data is significantly lower than predicted. However, because it is still positive slope, the conclusion is supported by the graph.

There is no literature value to which to compare the experimental data. Because the composition of sunflower seed oil is not consistent in terms of the fatty acids present in the triglyceride form, there is no theoretical value for its rate of oxidation at different temperatures. For this reason, percent error cannot be calculated. Because, in theory, increased heat should act as a catalyst for the oxidation of polyunsaturated oils as it did in the experimental trials, the results of the trials can be taken as relatively true. Again, because there is no literature value for this result, the accuracy of the solution with regard to percent error cannot be absolutely verified.

EV: Trying to relate to scientific context.

There are a few limitations in the execution of and materials used in the experiment:

First, although the aluminum foil around the beakers controlled the oils' exposure to light, it did not completely control exposure to the air. Because of this, each beaker may have had a different ratio of oxygen to oil. Less oxygen in the beakers would have led to a lower rate of oxidation, and more oxygen would have led to a higher rate of oxidation. This may have been the reason behind slight inconsistencies in some trials.

EV: If they were exposed to the same air (I assume they were) and the oils had the same surface area of interface with air, then this would have been constant.

Second, the places the beakers were stored over the 72 hr period had varying conditions. While the refrigerator and freezer were sealed while closed, they were opened and closed an unknown number of times, allowing for an unknown number of brief exposures to fresh air from outside. Beakers in the water baths, however, stayed in unsealed but fairly untouched

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environments. This difference in exposure to new air may have affected the results. If each exchange of air for the refrigerator increased the concentration of oxygen in the air, then the rate of oxidation of the oil may have increased.

C: If unsealed then surely there is constant interaction with environment. Not sure what the student is saying here.

Third, because the oil was stored at different temperatures, the reaction with potassium dichromate (IV) was carried out at different temperatures. There was residual heat from acidifying the potassium dichromate each time, but the reaction with 4 °C oil and 60 °C oil occurred at very different temperatures. A colder oil in the reaction may have slowed its speed or reduced its efficacy. This would have reduced the amount of color change, and thus the amount of absorption and the measured rate of oxidation.

EV: This should have been standardized. Samples should have been allowed to reach same thermal equilibrium prior to measurement.

The primary source of random error in this experiment was the uncertainty of the time of reaction. The clock used was accurate to the second, but the random error arose from the fact that there was up to .5 hr difference in the time the potassium dichromate (IV) reaction took place. This was simply due to the fact that the trials could not all be tested at once. Each datum in the final graph has a substantial % error attached to it for this reason.

Due to the lack of a literature value for the effect of storage temperature on oxidative rancidity, there is no final percent error. However, though the imprecision of the time measurement gave rise to random error, the data points in Figure 4 appear to be relatively close to the line of best fit, indicating that random error did not have a strong detrimental effect on the accuracy of the final data. However, although no percent error was calculated, it is highly likely that the uncontrolled aspects of the experiment gave rise to a significant amount of systematic error. Because of this, improvements to the experiments should be made to reduce systematic error more than random error.

EV: Reflecting on reliability of results.

The above mentioned systematic error and random error can be reduced or eliminated using the following improvements:

The issue of the imprecision of time measurement can be reduced by separating the three trials for each independent variable value. This way, only five tests are conducted at a time, which would significantly decrease the absolute uncertainty of the time measurement. this would ensure higher precision of the final conclusion.

The error stemming from the uncontrollable interruptions in the storage environment of the samples can be addressed in a number of ways. First, each beaker can be sealed with Parafilm, limiting the exchange of air between inside the beaker and outside the beaker. Second, the beakers can be placed in areas with less traffic in terms of opening and closing refrigerator doors.

EV: This will stop oxidation.

EV: Student not really understanding significance of errors.

Another source of systematic error that can be reduced is the issue surrounding the different temperatures of oil as it undergoes its reaction with potassium dichromate (IV). One way this can be improved is by controlling the temperature at which the chemicals react by having the reaction occur within a warm water bath. This would eliminate any discrepancies caused by the difference between cold oil and warm oil, since all trials would be heated to the same temperature.

EV: Good point.

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