

CHEM 133.02 LAB2-AC2

Experiment #4: Assessment of Canola Oil, Palm Oil, and CO-PO Mixture by Fourier Transform Infrared (FTIR) Spectroscopy and ^1H Nuclear Magnetic Resonance (^1H -NMR) Spectroscopy

Name of Students: CALUBAQUIB, Raphael Paulo; REYES, Frances Alissa; SANGUYO, Francis Miguel

Date Started: February 5, 2025

Date Completed: February 13, 2025

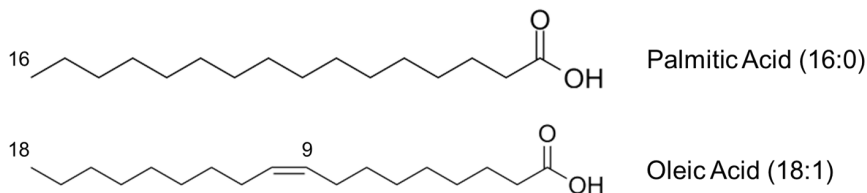
Introduction

Canola oil (CO) and palm oil (PO) are two commonly found examples of vegetable oils that are utilized in both food-related industries and research (Zambiazzi *et al.*, 2007). Like many vegetable oils, CO and PO are both composed of a mixture of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) with relatively limited nutritional value, low oxidative stability, and low physical and chemical stability (Hashempour-Baltork *et al.*, 2016). However, while CO is chiefly comprised of UFAs such as oleic acid (OLA), linoleic acid (LNA), and alpha-linolenic acid (ALA), most of PO is comprised of both SFAs such as palmitic acid and UFAs like OLA and LNA (Zambiazzi *et al.*, 2007). Table 1 and Figure 1 show the mole percentages and chemical structures of commonly-found SFAs and UFAs in CO and PO.

Table 1. Relative Mole Percentages of SFAs and UFAs in Canola and Palm Oils (Zambiazzi *et al.*, 2007)

Vegetable Oil	Saturated Fatty Acids (SFAs)			Unsaturated Fatty Acids (UFAs)			Overall FA Content	
	%14:0	%16:0	%18:0	%18:1 (OLA)	%18:2 (LNA)	%18:3 (ALA)	%SFA	%UFA
Canola Oil (CO)	0.06	3.75	1.87	62.41	20.12	8.37	6.98	93.02
Palm Oil (PO)	1.12	42.70	0.11	39.37	10.62	0.21	49.45	50.49

* Lipid numbers refer to the following: 14:0 = myristic acid; 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid (OLA); 18:2 = linoleic acid (LNA); 18:3 = alpha-linolenic acid (ALA)



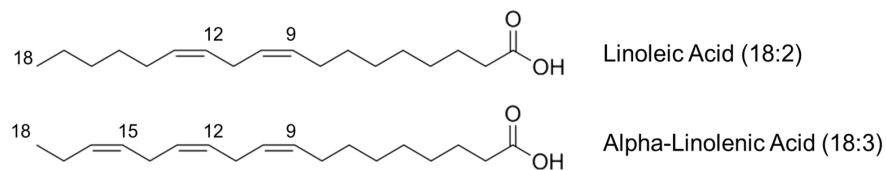


Figure 1. Chemical Structures of Palmitic, Oleic, Linoleic, and Alpha-Linolenic Acids

In many applications, CO and PO are commonly blended together to form various oil mixtures. In addition to having modified fatty acid compositions, these oil mixtures often have enhanced physical and chemical properties and functional characteristics such as frying stability (Hashempour-Baltork *et al.*, 2016).

As with other vegetable oils, the overall quality and composition of CO, PO, and CO-PO mixtures may be quantitatively assessed with instrumental techniques, two examples being Fourier Transform infrared spectroscopy (FTIR) and ^1H nuclear magnetic resonance spectroscopy (^1H -NMR). Depending on the relative composition of the oil, CO and PO possess notable bands in their FTIR absorption spectra, each of which correspond to structural features in their component FAs and/or their impurities (Ye & Meng, 2024). The composition of SFAs and various UFAs in pure CO and PO samples may also be quantitatively estimated from the areas of some structurally-relevant peaks found in their respective ^1H -NMR spectra with various mathematical models (Siudem *et al.*, 2022).

In this experiment, the relative fatty acid compositions of CO, PO, a 2:1 mixture of CO and PO (2C-1P), and a 1:2 mixture of CO and PO (1C-2P) was analyzed via FTIR from 300 to 4000 cm^{-1} in KBr salt and ^1H -NMR in CDCl_3 solvent. The wavenumbers and peak heights of FTIR signals and the chemical shift ranges and peak areas of ^1H -NMR signals were correlated to structural features and variations in the degree of unsaturation between the selected oils. The percentage of SFAs, UFAs, LNA, and ALA in CO, PO, and 2C-1P mixture were estimated from the peak areas obtained from their respective ^1H -NMR spectra.

Results

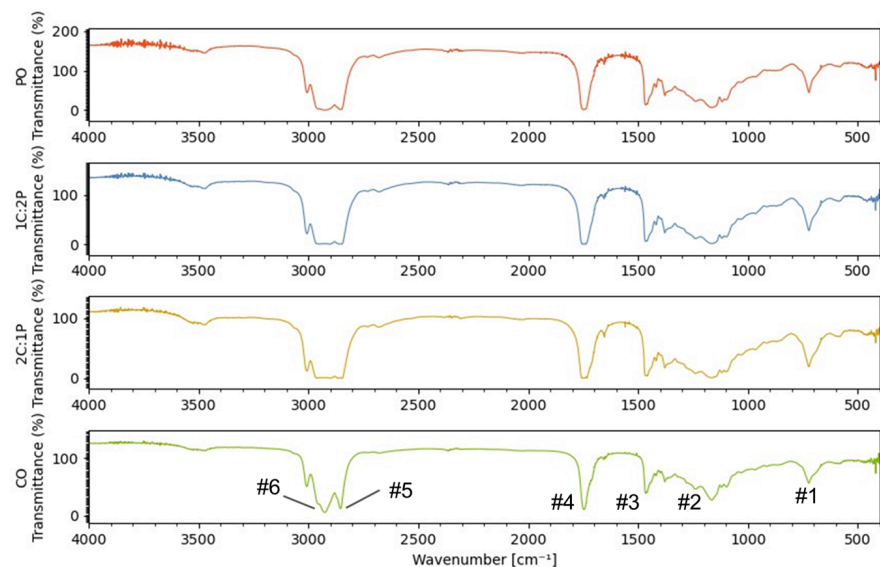


Figure 2. FTIR Spectra of CO, 2C:1P, 1C:2P, and PO in KBr Salt from 300 to 4000 cm^{-1} and their Peak Assignments from #1 to #6

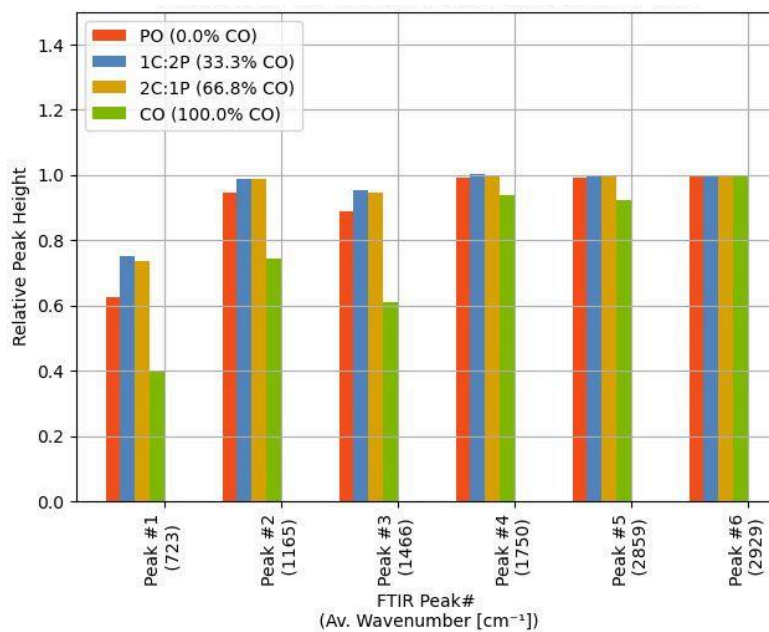


Figure 3. Peak Heights in the FTIR Spectra of CO, 2C:1P, 1C:2P, and PO Relative to Peak#6 at Around 2929 cm^{-1}

Table 2. Correlation of Relative Peak Heights (RPH) in the FTIR Spectra of CO, 2C-1P, 1C-2P, and PO to Mass% CO in Oil Mixtures

FTIR Peak#	FTIR Peak Range (cm ⁻¹)	Associated Molecular Vibration	RPH PO (0.0%)	RPH 1C-2P (33.3%)	RPH 2C-1P (66.8%)	RPH CO (100.0%)	Pearson Coefficient	Pearson p-Value
1	650-800	C-H Rocking	0.63	0.75	0.74	0.40	-0.55	0.45
2	1100-1250	Ester C-O Stretching	0.95	0.99	0.99	0.74	-0.67	0.33
3	1400-1500	Alkane C-H Bending	0.89	0.95	0.95	0.61	-0.67	0.33
4	1700-1800	Carbonyl C=O Stretching	0.99	1.00	1.00	0.94	-0.72	0.28
5	2800-2880	Alkane C-H Stretching	0.99	1.00	1.00	0.92	-0.71	0.29
6	2900-3050	Alkene =C-H Stretching	1.00*	1.00*	1.00*	1.00*	-	-

* Relative peak heights for peak #6 are set to 1.00 since they serve as the reference peaks in the calculation of RPH of the five other peaks.

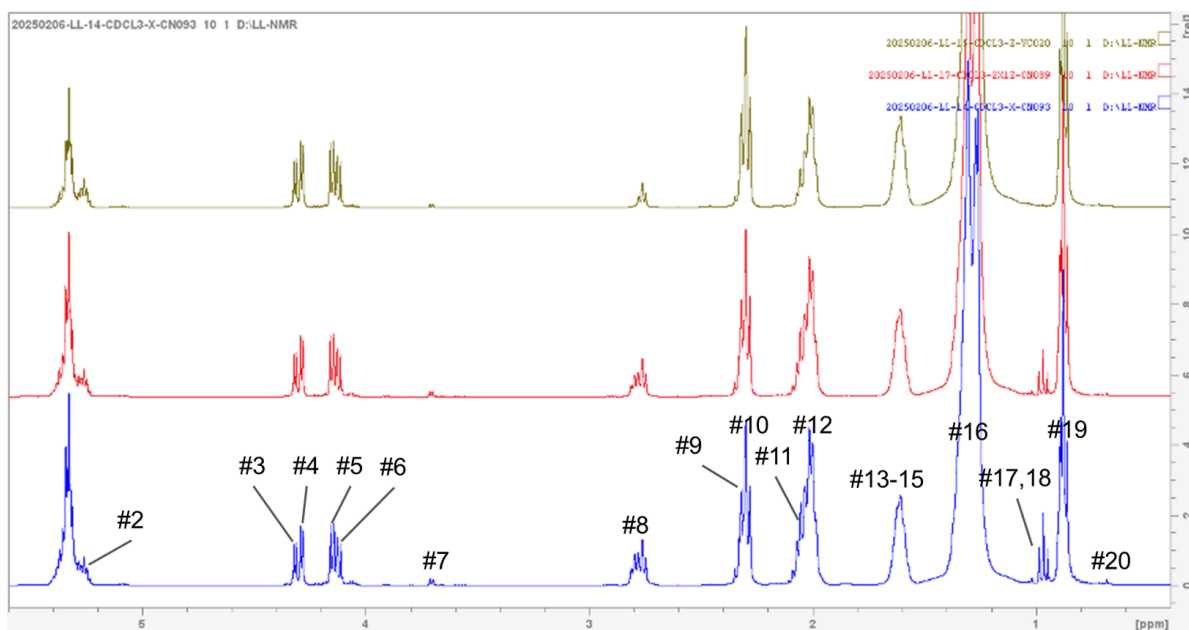


Figure 4. ¹H-NMR Spectra of PO (top, yellow), 2C-1P (middle, red), and CO (bottom, blue) in CDCl₃ Solvent from 0.4 to 6.6 δ and their Peak Assignments from #2 to #20 (Note that Peak #1 is not displayed within the given range)

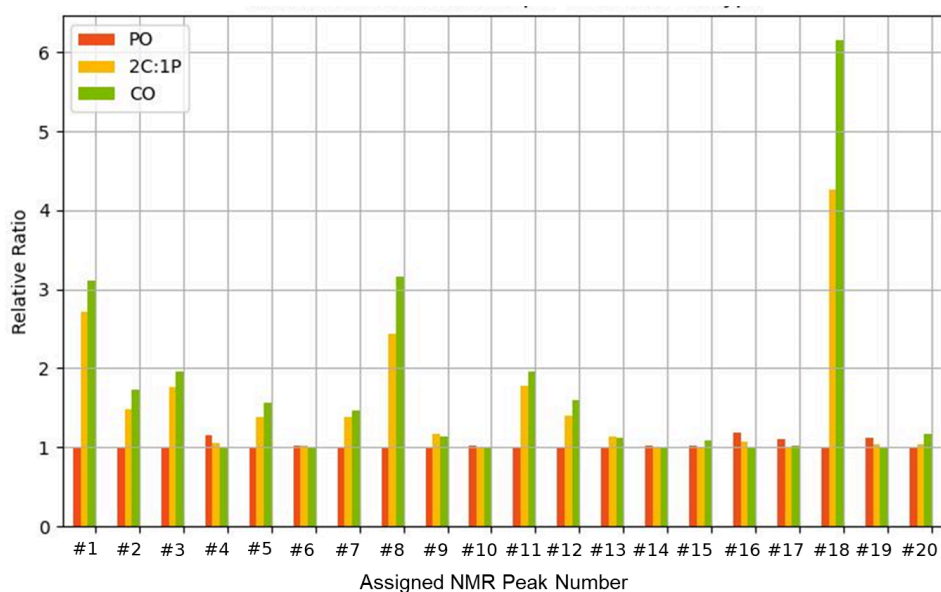


Figure 5. Relative Ratios of Peak Areas or Protons Corresponding to Each Assigned ^1H -NMR Peak in PO (orange), 2C-1P (yellow), and CO (green)

Table 3. Correlation of Relative Peak Areas (%) in the ^1H -NMR Spectra of CO, 2C-1P, and PO to Mass% CO in Oil Mixtures for Peaks with Statistically Significant Trends

NMR Peak#	Chemical Shift Range (δ)	Group Involved	FAs Present*	RPA PO (0.0%)	RPA 2C-1P (66.8%)	RPA CO (100%)	Pearson Coeff.	Pearson p-Value	Sign of Trend
2	5.22-5.43	Alkene =CH	UFAs	4.72	7.03	8.159	0.99999	0.003	(+)
4	4.26-4.34	Ester -CH ₂ -	TAGs	1.78	1.64	1.56	-0.9995	0.021	(-)
5	4.18-4.26	Ester -CH ₂ -	TAGs	0.0917	0.127	0.143	0.9997	0.017	(+)
8	2.72-2.84	Allylic -CH ₂ - Next to 2 Alkenes	LNA, ALA	0.696	1.70	2.20	> 0.99999	< 0.001	(+)
10	2.25-2.35	Acid -CH ₂ -	UFAs	5.68	5.58	5.53	-0.9999	0.010	(-)
12	1.94-2.09	Allylic -CH ₂ - Next to 1 Alkene	UFAs	6.38	8.91	10.2	0.99997	0.005	(+)

Table 3. Correlation of Relative Peak Areas (%) in the ¹H-NMR Spectra of CO, 2C-1P, and PO to Mass% CO in Oil Mixtures for Peaks with Statistically Significant Trends (continued)

NMR Peak#	Chemical Shift Range (δ)	Group Involved	FAs Present*	RPA PO (0.0%)	RPA 2C-1P (66.8%)	RPA CO (100%)	Pearson Coeff.	Pearson p-Value	Sign of Trend
16	1.18-1.41	Alkane -CH ₂ -	All FAs	58.9	52.9	49.8	-0.99997	0.005	(-)
18	0.93-1.00	-CH ₃ Next to Allylic -CH ₂ -	All FAs	0.161	0.686	0.993	0.9991	0.027	(+)
19	0.83-0.93	-CH ₃	All FAs Except LNA	8.65	8.02	7.71	-0.99998	0.004	(-)

* Legend: FA = fatty acid; UFA = unsaturated fatty acid; LNA = linoleic acid; ALA = alpha-linolenic acid; TAG = triacylglycerol

Table 4. Summary of Estimated Oil Fatty Acid Profile of CO, PO, and 2C-1P Based on the Peak Areas of Their Respective ¹H NMR Spectra

Sample	LNA Content (%)*	ALA Content (%)*	OLA Content (%)*	UFA Content (%)*	SFA Content (%)*
CO	0.114	0.873	1.99	2.98	97.0
2C-1P	0.0788	0.693	1.89	2.66	97.3
PO	0.0183	0.312	1.68	2.01	98.0
Expected Values for 2C-1P	0.0821	0.686	1.89	2.65	97.3

* Estimated values for LNA, ALA, OLA, UFA, and SFA content in the three oil samples were calculated based on a method reviewed by Siudem *et al.* (2022).

Discussion

The FTIR spectra of the oil samples PO, 1C-2P, 2C-1P, and CO, arranged in order of increasing CO content, from 300 to 4000 cm^{-1} and their respective peak assignments are summarized in Figure 2. The relationship between the ratios of the heights of the FTIR peaks to peak #6 of each given spectrum and the mass %CO of each oil is described in Figure 3 and Table 2. From Figure 2, it was observed that all four spectra generally had similar shapes and exhibited six FTIR peaks, five of which correspond to the vibrations of alkene C-H, alkane C-H, and acid C=O bonds present in the structures of component SFAs and UFAs in Figure 1. These features are consistent with the findings of Ye and Meng (2022), as these six FTIR peaks are commonly found in other edible fats and oils. Additionally, the presence of peaks #6 and #2 in all four spectra indicated significant amounts of UFAs in both PO and CO, which is consistent with the oil composition data by Zambiazzi *et al.* (2007) (Figure 1).

Comparison of relative heights of FTIR peaks in Figure 3 showed that the difference in the relative heights of FTIR peaks to that of peak #6, corresponding to alkene C-H vibrations in UFAs, is generally larger in CO than in PO. However, all peaks but peak#1 had similar heights to each other in the oil mixtures 1C-2P and 2C-1P. The former observation qualitatively reflects the larger UFA content in CO (93.02%) than in PO (50.49%). Nevertheless, the two oil mixtures, of which have overall FA compositions intermediate that of CO and PO, did not exhibit peaks of intermediate heights. One reason is that both oils were unable to completely and homogeneously mix during sample preparation. This resulted in the effects of IR absorptions of SFAs and UFAs to be additive, rather than averaged by FA composition. Furthermore, it was found from Table 2 that there were only weak, statistically insignificant, and negative correlations between the mass %CO of each oil and the relative heights of FTIR peaks ($r = -0.55, -0.67, -0.67, -0.72, -0.71$). This analysis showed that while FTIR was able to identify the general structural features of all four oils and qualitatively suggest differences in unsaturation between pure oils like CO and PO, it was unable to reliably relate the peak signals with the composition of pure and mixed oils combined. According to Ye and Meng (2022), simple mathematical relationships involving the FTIR spectra of different oils cannot be developed due to variances in oil composition.

The ^1H -NMR spectra of PO, 2C-1P, and CO from 0.4 to 6.6 δ and their respective peak assignments are shown in Figure 4. The relative ratios of relative peak areas exhibited by each NMR peak are compared between the three oils in Figure 5, where a ratio of “1” represents the smallest peak given by any of the oils. Finally, Table 3 summarizes statistically significant relationships between the relative peak areas of each NMR peak and the mass% CO of the respective oil. The ^1H -NMR spectra of the three oils have the same number of peaks at 20, have similar ranges for the chemical shifts of each peak, and generally have similar overall features. Following the classification scheme proposed by Siudem *et al.* (2022), some of these signals such as peaks #2, 8, and 12 indicated the presence of protons that are within the vicinity of an unsaturated olefinic region in UFAs. Other signals such as peaks #16, 18, and 19 referred to alkane protons with similar environments across most, if not all FAs found in the three oils.

Finally, peaks #4 and 5 were associated with protons next to an ester group, which suggested the presence of triacylglycerols.

The bar chart in Figure 5, however, suggested that the relative areas of nine peaks in the ^1H -NMR spectra significantly differ and follow a certain trend when compared across the three oils. The statistical analysis shown in Table 3 confirmed that the areas of peaks #2, 5, 8, 12, and 18 followed strong, linear positive trends with mass% CO, whereas the areas of peaks #4, 10, 16, and 19 followed strong, linear negative trends with mass% CO. The relationships for these nine peaks yielded r values above 0.9991 and were considered statistically significant at 5% significance ($p < 0.021$).

These observations strongly suggested that, unlike FTIR, the ^1H -NMR spectra of the different oils may be correlated quantitatively with their FA compositions on the basis of their peak areas. As the mass% CO of the oil sample is raised, the proportion of UFAs relative to SFAs significantly increases from 50.49% to 93.02% (Zambiasi *et al.*, 2007). Compared to PO, the proportion of alkene, allylic, and methyl protons in the vicinity of olefinic regions was raised in CO, as reflected by the positive trends of peaks #8, 12, and 18. As an example, the area of peak #8, which was a peak yielded exclusively by allylic protons in ALA and LNA, was raised from 0.696% in PO to 2.20% in CO. This suggested that CO had 3.16 times more ALA and LNA than PO, compared to the literature value of 2.63 by Zambiasi *et al.* (2007).

However, as indicated by the areas of peak #16, the proportion of alkane $-\text{CH}_2-$ protons outside olefinic regions was lowered from 58.9% in PO to 49.8% in CO. It can be further estimated that FAs in CO have more 0.62 C=C bonds per molecule on average than PO. This is consistent with Zambiasi *et al.* (2007), which yielded a value of 0.67 C=C bonds per molecule. From these trends, the ^1H -NMR spectra of CO and PO was able to give a quantitative estimate on the relative degree of unsaturation and the chemical structures of the two oils. Furthermore, mathematical models linking the UFA content in oils to ^1H -NMR peak areas may be constructed. A similar finding across different vegetable oils was explored by Siudem *et al.* (2022).

One such model reviewed by Siudem *et al.* (2022) was utilized to estimate the %LNA, %ALA, %OLA, %UFA, and %SFA content of CO, 2C-1P, and PO. The calculated values, which were estimated based on the areas of peaks #2, 8, 12, 18, and 19, were summarized in Table 4. The ratios of the values followed similar trends as the ^1H -NMR peak areas, which further suggested the increased UFA content of CO relative to PO and confirmed the 2:1 ratio of CO and PO in 2C-1P. Nevertheless, the estimated %UFA values at 2.98%, 2.66%, and 2.01%, respectively, significantly deviated from the expected values by Zambiasi *et al.* (2007). A similar case was found for the computed %LNA, %ALA, %OLA, and %SFA content for the three oils. This showed that the ^1H -NMR spectra of the oils cannot successfully give estimates on their FA composition under the method performed for this experiment. One reason is that the oil sample was not separated into its components beforehand and was directly measured by the instrument for its ^1H -NMR spectra. As with the case of FTIR, it is possible that the interactions between

different FAs might have altered the signals and peak areas for protons on adjacent molecules during measurement. As a result, the accuracy of calculating peak areas of different oil components might have been significantly compromised, making direct calculations on FA composition inaccurate (Siudem *et al.*, 2022). Another reason is that the oil samples might have degraded under heat and sunlight or mixed with oily contaminants to a noticeable extent during the sample preparation stage. These processes might have introduced various components in the samples which yielded small, interfering peaks in the oils' FTIR and ¹H-NMR spectra, leading to more noisy signal measurements, compromised peak shapes, and less accurate computations for %FA content (Ye & Meng, 2022).

Calculations

I. Calculations in Computing %CO in CO, 1C-2P, 2C-1P, and PO:

$$\text{m\%CO [PO]} = 0.0\%$$

$$\text{m\%CO [1C - 2P]} = \left(\frac{0.5046 \text{ g CO}}{0.5046 \text{ g CO} + 1.0088 \text{ g PO}} \right) \times 100\%$$

$$\text{m\%CO [1C - 2P]} = 33.3\%$$

$$\text{m\%CO [2C - 1P]} = \left(\frac{1.0308 \text{ g CO}}{1.0308 \text{ g CO} + 0.5125 \text{ g PO}} \right) \times 100\%$$

$$\text{m\%CO [2C - 1P]} = 66.8\%$$

$$\text{m\%CO [CO]} = 100.0\%$$

II. Calculations for Analysis of Relative Peak Heights in FTIR and Peak Areas in ¹H-NMR

The calculations for analyzing, plotting and correlating relative peak heights in the FTIR spectra and relative peak areas in the ¹H-NMR spectra of the four vegetable oils used in this experiment were performed by a computer program developed by Sanguyo (2025) using the programming language Python 3.7. The program and CSV files may be accessed below:

Link to Program Used for Computations (Sanguyo, 2025)

<https://github.com/NotAMadTheorist/FTIR-and-1H-NMR-Analysis-of-Cooking-Oils>

Link to CSV File for FTIR Peak Heights (Sanguyo, 2025)

https://github.com/NotAMadTheorist/FTIR-and-1H-NMR-Analysis-of-Cooking-Oils/blob/main/FTIR_AllPeakCorrelations.csv

Link to CSV File for ¹H-NMR Relative Peak Areas (Sanguyo, 2025)

https://github.com/NotAMadTheorist/FTIR-and-1H-NMR-Analysis-of-Cooking-Oils/blob/main/1H-NMR_AllPeakCorrelations.csv

III. Calculations for Estimating Difference in # of C=C Bonds between Canola and Palm Oils

$$\% \text{ decrease area \#16} = \left(\frac{58.9 - 49.8}{58.9} \right) \times 100\%$$

$$\% \text{ decrease area \#16} = 15.4499\%$$

$$\% \text{ decrease alkane H} = 15.4499\%$$

Assuming that the fatty acid chain has 18 carbons, at most 16 carbons have $-\text{CH}_2-$ groups. Hence the chain will have at most $16 \times 2 = 32$ alkane H.

$$\# \text{ lost alkane H} = 32(0.154499)$$

$$\# \text{ lost alkane H} = 4.9440$$

For every C = C bond introduced, $4 + 2 + 2 = 8$ alkane protons change in environment. Therefore, they won't count to area#16, and the average # C = C bonds introduced is:

$$\# (\text{C} = \text{C}) = \frac{4.9440}{8}$$

$$\# (\text{C} = \text{C}) = 0.62 \text{ double bonds introduced}$$

The literature value may be computed from Table 1 as follows:

Suppose you have 100 moles of canola oil and palm oil.

$$\text{mol (C} = \text{C) in CO} \approx 62.41(1) + 20.12(2) + 8.37(3)$$

$$\text{mol (C} = \text{C) in CO} \approx 127.76 \text{ mol}$$

$$\text{mol (C} = \text{C) in PO} \approx 39.37(1) + 10.62(2) + 0.21(3)$$

$$\text{mol (C} = \text{C) in PO} \approx 61.24 \text{ mol}$$

$$\# (\text{C} = \text{C}) \text{ lit value} = \frac{127.76 \text{ mol} - 61.24 \text{ mol}}{100 \text{ mol}}$$

$$\# (\text{C} = \text{C}) \text{ lit value} = 0.67 \text{ double bonds introduced}$$

IV. Estimation of Fatty Acid Content in Canola and Palm Oils via $^1\text{H-NMR}$ Peak Areas

The calculations for computing %LNA, %ALA, %OLA, %UFA, and %SFA in canola oil, palm oil, and 2:1 CO-PO mixture were performed in the following spreadsheet. The formulas used were adapted from page 2 of the PDF version of Siudem *et al.* (2022).

Link to Spreadsheet for %FA Calculations

<https://docs.google.com/spreadsheets/d/1CT-A5z1LZpV97sRaM8uoqI8yHdTRKxMD/edit?usp=sharing&ouid=115501014274357653039&rtpof=true&sd=true>

Conclusion

Four vegetable oil samples, including canola oil (CO), palm oil (PO), a 2:1 mixture of CO and PO (2C-1P), and a 1:2 mixture of CO and PO (1C-2P) were directly analyzed for their structure and relative compositions via FTIR in KBr and $^1\text{H-NMR}$ in CDCl_3 solvent. Results showed that the FTIR spectra of all four oils yielded six common peaks or absorptions with differing peak heights, and differences in degree of unsaturation or UFA content only between pure CO and PO. Only weak, negative trends ($|r| < 0.72$) were found between FTIR peak heights and mass% CO. The $^1\text{H-NMR}$ spectra of CO, PO, and 2C-1P yielded twenty common peaks from 0.4 to 6.6 δ , nine of which were found to have statistically significant, linear trends ($|r| > 0.999$) with the mass% CO of each oil. From those trends, it was estimated that CO has 3.16 times more linoleic acid (LNA) and alpha-linolenic acid (ALA) content and had additional 0.62 C=C bonds per molecule on average compared to PO. However, the fatty acid composition of the three oils were not accurately determined from the peak areas. Some notable sources of error included interaction effects between different components within a pure oil or oil mixture and sample degradation due to heat or light. As such, it is recommended that future experiments explore the spectrochemical behavior of vegetable oils further by verifying trends in FTIR and $^1\text{H-NMR}$ spectra obtained from this study in other vegetable oils, and employing spectrochemical methods for vegetable oils preceded by separation in GC and/or LC.

References

- Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S., & Savage, G. P. (2016, November). *Vegetable oil blending: A review of physicochemical, nutritional and health effects*. ScienceDirect. <https://www.sciencedirect.com/science/article/abs/pii/S0924224416302886>
- Sanguyo, F. (2025). FTIR and 1H-NMR Analysis of Cooking Oils [Computer program]. Ateneo de Manila University. <https://github.com/NotAMadTheorist/FTIR-and-1H-NMR-Analysis-of-Cooking-Oils>
- Siudem, P., Zielinska, A., Paradowska, K. (2022). Application of 1H NMR in the study of fatty acids composition of vegetable oils. *Journal of Pharmaceutical and Biomedical Analysis*, 212, 114568. <https://doi.org/10.1016/j.jpba.2022.114658>
- Ye, Q., Meng, X. (2022). Highly efficient authentication of edible oils by FTIR spectroscopy coupled with chemometrics. *Food Chemistry*, 385, 132661. <https://doi.org/10.1016/j.foodchem.2022.132661>
- Zambiazi, R., Przybylski, R., Zambiazi, M., Mendonca, C. (2007). Fatty Acid Composition of Vegetable Oils and Fats. *Boletim do Centro de Pesquisa de Processamento de Alimentos*, 25(1), 111-120. <https://doi.org/10.5380/CEP.V25I1.8399>