Mini Project 2

Jackson Passey, Gavin Hatch, Ty Hawkes

2025-10-20

Introduction

This analysis attempts to answer questions about fatty acids in olives found in two different regions. The data includes response variables palmitic, palmitoleic, stearic, oleic, linoleic, eicosanoic, linolenic, and eicosenoic acids predicted by region (Region 2 and Region 4).

This report assesses whether the olive samples from each region deviate from their historical averages and whether the two regions deviate from each other. We also explore whether linoleic and linolenic acids can be dropped from the list of fatty acids without significantly decreasing the separation of the two samples.

The data analyzed contains several missing values. Part of this report determines whether the data is missing at random and provides a new dataset with imputed values using the Expectation-Maximization (EM) algorithm and multiple imputation.

We will show that the olive samples from both regions deviate significantly from their historical averages and that the two regions differ significantly from each other in terms of their fatty acid profiles.

Data Preparation

The data for regions 2 and 4 were obtained from separate organizations, which may have used different data collection procedures. Both datasets contain missing values in various fatty acid measurements. In order to perform a comprehensive multivariate analysis, these missing values need to be addressed. It is assumed prior to this analysis that the data from both regions are distributed multivariate normal.

Under the assumption that the data is missing at random (MAR), we employed the Expectation-Maximization (EM) algorithm combined with multiple imputation to fill in the missing values. Expectation-Maximization is an iterative method that estimates the parameters of a statistical model in the presence of missing data. The algorithm alternates between estimating the missing values given the observed data (E-step) and updating the model parameters based on the complete data (M-step). This process continues until convergence. The algorithm can be summarized as follows:

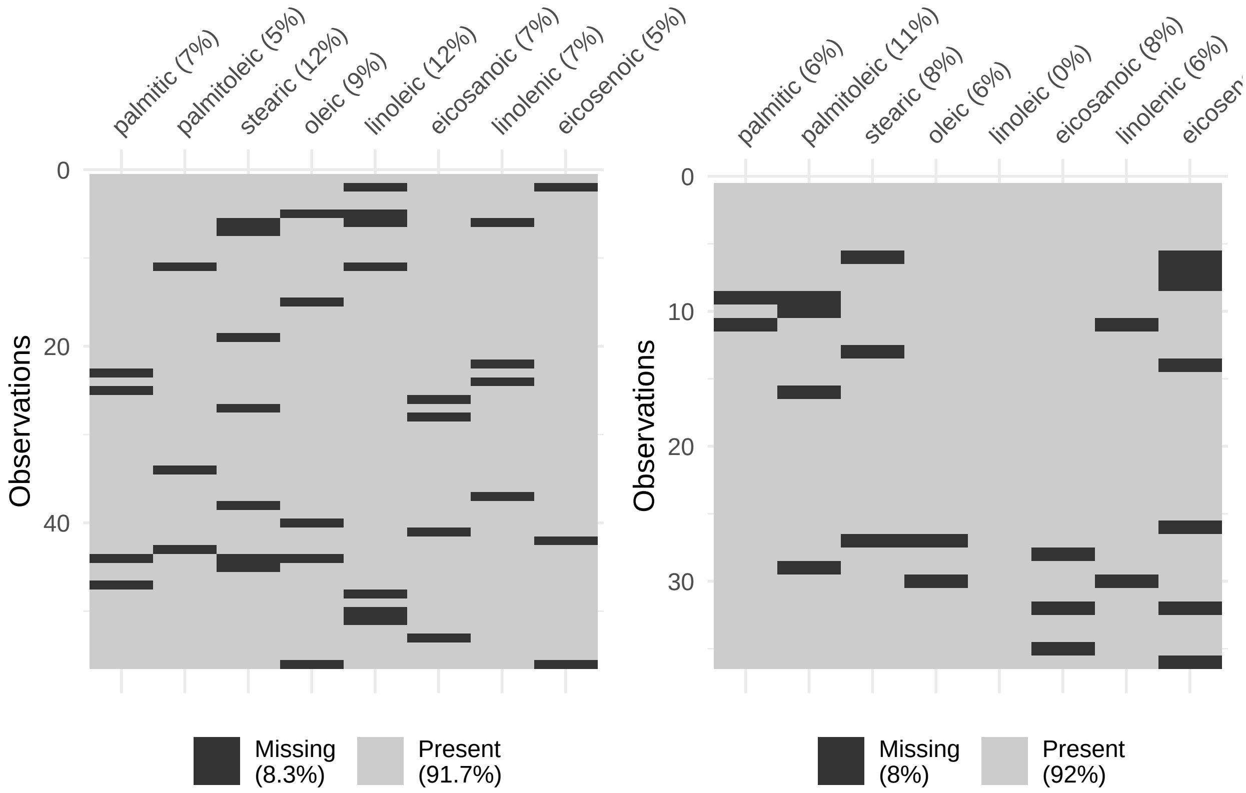
$$\text{E-step: } Q(\theta|\theta^{(t)}) = E[\log L(\theta; X_{obs}, X_{mis})|X_{obs}, \theta^{(t)}]$$

After convergence, multiple imputation is performed by generating several complete datasets, each with different imputed values drawn from the estimated distribution of the missing data. This approach accounts for the uncertainty associated with the imputation process. This is done by adding a random error term to each imputed value based on the estimated covariance structure of the data.

Assessment of Missing at Random (MAR) Assumption

In order to use the EM algorithm and multiple imputation correctly, we must first assess whether the missing data mechanism is consistent with the Missing at Random (MAR) assumption. MAR assumes that the probability of missingness is related to the observed data but not to the unobserved data. To test this assumption, we examineed the patterns of missingness in the datasets for region 2 and region 4 visually and statistically with fisher's exact p-values.

```
## Warning: package 'gridExtra' was built under R version 4.3.2
```



An initial look at the missing data patterns for both regions indicates that the missingness does not appear to be systematically related to any particular variable. The visualizations show that missing values are scattered throughout the datasets without any obvious patterns.

To assess the MAR assumption more formally, we conducted pairwise Fisher's exact tests between the missingness indicators of each pair of variables. Fishers exact tests use contingency tables to determine if there are nonrandom associations between two categorical variables. In this case, we created 2x2 tables for each pair of variables, where one variable indicates whether a value is missing (1) or observed (0) for the dependent variable, and the other variable indicates the same for the predictor variable. The null hypothesis for each test is that the missingness of the dependent variable is independent of the missingness of the predictor variable.

The resulting p-values for each unique pair of variables (shown in the appendix) were all above common significance thresholds (e.g., 0.05), suggesting that there is no strong evidence to reject the MAR assumption. Based on these findings, we proceed under the assumption that the data is missing at random (MAR), allowing us to utilize the EM algorithm and multiple imputation for handling missing values in our multivariate analyses.

Analysis for Region 2

For Region 2, the sample mean vector was compared to the historical average

$$\boldsymbol{\mu}_{02} = (1300,\ 120,\ 265,\ 7310,\ 820,\ 45,\ 65,\ 28),$$

representing the eight fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, eicosanoic, linolenic, and eicosenoic). Using the EM algorithm followed by multiple imputation to address missing data, a multivariate hypothesis test was performed. For each imputed dataset, the sample mean vector and within-imputation covariance matrix were computed. These results were then combined using Rubin's rules to form the following:

$$\bar{\boldsymbol{\theta}} = \frac{1}{M}\sum_{m=1}^{M}\boldsymbol{\theta}_m, \quad \mathbf{U}_{\text{bar}} = \frac{1}{M}\sum_{m=1}^{M}\mathbf{U}_m, \quad \mathbf{B} = \frac{1}{M-1}\sum_{m=1}^{M}(\boldsymbol{\theta}_m - \bar{\boldsymbol{\theta}})(\boldsymbol{\theta}_m - \bar{\boldsymbol{\theta}})^\top.$$

The total covariance matrix, incorporating both within- and between-imputation variability, was then

$$\mathbf{T} = \mathbf{U}_{\text{bar}} + \left(1 + \frac{1}{M}\right)\mathbf{B}.$$

Using $\bar{\boldsymbol{\theta}}$ and $\mathbf{T}$, a pooled Hotelling's T^2 statistic was computed as

$$T^2 = n(\bar{\boldsymbol{\theta}} - \boldsymbol{\mu}_0)^\top \mathbf{T}^{-1}(\bar{\boldsymbol{\theta}} - \boldsymbol{\mu}_0),$$

which follows an approximate F-distribution under the null hypothesis $\mathcal{H}_0 : \boldsymbol{\mu} = \boldsymbol{\mu}_0$. For Region~2, this test produced

$$T^2 = 511.44, \quad F = 55.79, \quad p < 0.001.$$

The extremely small p-value provides strong evidence that the Region~2 sample mean vector significantly deviates from its historical average. The pooled mean estimates after combining across imputations were

$$\bar{\boldsymbol{\theta}} = (1299.87,\ 121.42,\ 264.44,\ 7310.76,\ 817.79,\ 45.72,\ 63.63,\ 28.40).$$

Examination of the sample means relative to the hypothesized values suggests that most components are close to their historical levels, but small shifts across several variables collectively contribute to the multivariate difference. In particular, *oleic* and *linoleic acids* exhibit the largest deviations, consistent with their large variances and strong negative covariance pattern, suggesting systematic changes in fatty-acid composition for Region~2.

Analysis for Region 4

For Region~4, the sample mean vector was compared to the historical average

$$\boldsymbol{\mu}_{04} = (1230,\ 105,\ 275,\ 7360,\ 830,\ 41,\ 75,\ 38),$$

using the same EM–multiple-imputation procedure described above. The resulting combined multivariate test yielded

$$T^2 = 495.93, \quad F = 49.59, \quad p < 0.001.$$

The test again provides overwhelming evidence that the Region~4 fatty-acid profile differs significantly from its historical mean vector. The pooled mean estimates

$$\bar{\boldsymbol{\theta}} = (1230.03,\ 105.36,\ 273.61,\ 7358.55,\ 834.72,\ 42.36,\ 75.83,\ 37.69)$$

are remarkably close to the hypothesized values, indicating that while the overall multivariate deviation is statistically significant, the practical differences are small in magnitude.

Comparison between Regions 2 and 4

To compare whether Regions 2 and 4 are the same, we used Hotelling's 2-Sample T test. We calculated a T^2 value of 189.7534332 which converts to an F-Statistic of 21.8743541. Using the F distribution with numerator degrees of freedom of 8 and a denominator degrees of freedom of 83 results in a P-Value of <.0001.

This is statistically significant and provides overwhelming evidence that the overall fatty-acid profiles of regions 2 and 4 differ. The agronomist's belief that region 2 and region 4 olives have evolved to have essentially the same profile in terms of the eight fatty acids is not supported by the data. The observed differences are unlikely to have come from random sampling variation alone.

It is important to note that the two samples were drawn by different organizations with potentially different data collection procedures, chemical analysis tools, and data censoring mechanisms. This combined with missing data that was imputed using MVI exposes limitations of this analysis. We do not know if significant variation comes from the different data collection procedures.

```
##      Variable Coefficient
## 1    palmitic -0.07663898
## 2 palmitoleic -0.12250415
## 3     stearic -0.18522911
## 4       oleic -0.10728903
## 5    linoleic -0.11471857
## 6  eicosanoic  0.51834314
## 7   linolenic -0.74245026
## 8  eicosenoic -0.31658504
```

Looking at the discriminant function, we can see that the largest coefficients are for Linolenic, Eicosanoic, and Eicosenoic acids (in that order). This suggests that these fatty acids contribute the most to the difference between regions 2 and 4. Further investigation into these specific fatty acids may provide more insights into the differences in olive profiles between the two regions.

Assessment of Linoleic and Linolenic Acids

```
##                    [,1]
## palmitic     0.04397878
## palmitoleic  0.07029822
## stearic      0.10629253
## oleic        0.06156712
## linoleic     0.06583051
## eicosanoic  -0.29744787
## linolenic    0.42605030
## eicosenoic   0.18167029
```

```
##                  [,1]
## palmitic     5.339851
## palmitoleic  2.453354
## stearic      4.319048
## oleic       14.405667
## linoleic     7.832097
## eicosanoic  -1.765838
## linolenic    3.781679
## eicosenoic   1.241544
```

Using a conditional Wilk's Λ test, we assessed whether linoleic acid and linolenic acid were important in contributing to the significant difference observed between regions 2 and 4. We set up the following hypothesis:

CHECK THESE HYPOTHESES

$$H_0 : \boldsymbol{\mu}^{(full)} = \boldsymbol{\mu}^{(reduced)}$$

$$H_a : \boldsymbol{\mu}^{(full)} \neq \boldsymbol{\mu}^{(reduced)}$$

Where the full model includes all 8 fatty acids and the reduced model excludes linoleic and linolenic acids.

With a Wilk's Λ of 0.5948317 converted to an F statistic, we calculated a P-value of $< .05$ and reject the null hypothesis. To support this rejection, we found that the discriminant functions separating regions also indicate some form of separation for linoleic and linolenic acids. In this case, we see that the standardized discriminant scores for each of them have high positive influence indicating that observations with higher values of linoleic and linolenic acids more likely reside in Region 2.

We conclude that linoleic and linoeic acids are important in contributing to the separation of the two regions beyond the information available from the other 6 acids.

Conclusions and Recommendations

The missing data appeared consistent with the MAR assumption, supporting the use of the EM algorithm followed by multiple imputation to generate valid inferences. For Region~2, the pooled Hotelling's $T^2 = 511.44$ ($F = 55.79$, $p < 0.001$) provided strong evidence that the sample mean vector differed from its historical average. The estimated means (1299.87, 121.42, 264.44, 7310.76, 817.79, 45.72, 63.63, 28.40) suggest that although most fatty acids were near their expected values, coordinated shifts—especially in oleic and linoleic acids—produced a significant overall difference.

Similarly, Region~4 showed $T^2 = 495.93$ ($F = 49.59$, $p < 0.001$), with estimated means (1230.03, 105.36, 273.61, 7358.55, 834.72, 42.36, 75.83, 37.69). The deviations, though modest for individual components, combined to indicate a multivariate shift in fatty acid composition relative to historical levels.

The multivariate analysis comparing regions 2 and 4 revealed strong evidence that the fatty acid profiles differ between the two regions. Specifically, Hotelling's two-sample T^2 test indicated a statistically significant difference leading us to reject the null hypothesis of equal mean vectors. This suggests that olives from regions 2 and 4 possess distinct chemical compositions in terms of their fatty acid content.

To further understand which variables contributed most to this separation, a discriminant function was constructed. The resulting discriminant coefficients indicated that linolenic, eicosanoic, and eicosenoic acids were the strongest contributors to the separation of regions. In addition, our assessment of linoleic and linolenic acids confirmed their importance in contributing to the observed differences.

Limitations: Limitations of this analysis include the fact that we do not know any bias that may come from collection techniques between two different organizations. Also, missing data adds unknowns to the analysis.

Future Work: Further investigation into specific fatty acids like linolenic, eicosanoic, and eicosenoic acids may provide more insights into the differences in olive profiles between the two regions. Additionally, looking at other regions (e.g. 1 and 3) could provide additional information on regional differences. Finally, it would be helpful to look into the source of the missingness in the data.

Appendix

```r
pairwise_missing_fisher_p_values(oliver2a)
```

```
##             palmitic palmitoleic stearic  oleic linoleic eicosanoic linolenic
## palmitic          NA      1.0000  0.4231 0.3196   1.0000          1    1.0000
## palmitoleic   1.0000          NA  1.0000 1.0000   0.3354          1    1.0000
## stearic       0.4231      1.0000      NA 0.5008   1.0000          1    0.4231
## oleic         0.3196      1.0000  0.5008     NA   0.5008          1    1.0000
## linoleic      1.0000      0.3354  1.0000 0.5008       NA          1    0.4231
## eicosanoic    1.0000      1.0000  1.0000 1.0000   1.0000         NA    1.0000
## linolenic     1.0000      1.0000  0.4231 1.0000   0.4231          1        NA
## eicosenoic    1.0000      1.0000  1.0000 0.2487   0.3354          1    1.0000
##             eicosenoic
## palmitic        1.0000
## palmitoleic     1.0000
## stearic         1.0000
## oleic           0.2487
## linoleic        0.3354
## eicosanoic      1.0000
## linolenic       1.0000
## eicosenoic          NA
```

```
pairwise_missing_fisher_p_values(oliver4a)
```

```
##             palmitic palmitoleic stearic  oleic eicosanoic linolenic eicosenoic
## palmitic          NA      0.2127  1.0000 1.0000     1.0000    0.1095     1.0000
## palmitoleic   0.2127          NA  1.0000 1.0000     1.0000    1.0000     0.5658
## stearic       1.0000      1.0000      NA 0.1619     1.0000    1.0000     0.4882
## oleic         1.0000      1.0000  0.1619     NA     1.0000    0.1095     1.0000
## eicosanoic    1.0000      1.0000  1.0000 1.0000         NA    1.0000     0.4882
## linolenic     0.1095      1.0000  1.0000 0.1095     1.0000        NA     1.0000
## eicosenoic    1.0000      0.5658  0.4882 1.0000     0.4882    1.0000         NA
```

```
hotellings_with_imputed_datasets(oliver2a_imputed, mu2a_not)
```

```
## $mean
##    palmitic palmitoleic     stearic       oleic    linoleic  eicosanoic 
##  1300.06030   121.33160   264.12679  7310.78522   818.14086    45.65906 
##   linolenic  eicosenoic 
##    63.66005    28.38470 
## 
## $total_variance
##                 palmitic palmitoleic     stearic       oleic    linoleic
## palmitic      77.8875051   3.3149380   0.7506785 -111.356458   28.164103
## palmitoleic    3.3149380   7.6833418  -4.4639117   -1.191266   -5.688477
## stearic        0.7506785  -4.4639117  23.0608989  -20.023208    1.344744
## oleic       -111.3564576  -1.1912663 -20.0232085  308.780118 -165.309633
## linoleic      28.1641033  -5.6884769   1.3447440 -165.309633  138.141238
## eicosanoic    -0.4985391  -0.5174102   0.3639924   -3.020398    2.545361
## linolenic      1.2830179   0.1058002  -0.9230624   -4.613957    2.441765
## eicosenoic    -0.6317896  -0.1832070   0.1128521   -2.728616    2.425766
##             eicosanoic  linolenic eicosenoic
## palmitic    -0.4985391  1.2830179 -0.6317896
## palmitoleic -0.5174102  0.1058002 -0.1832070
## stearic      0.3639924 -0.9230624  0.1128521
## oleic       -3.0203985 -4.6139573 -2.7286161
## linoleic     2.5453611  2.4417654  2.4257662
## eicosanoic   0.7848516  0.2804718  0.2579265
## linolenic    0.2804718  0.8444362  0.2977100
## eicosenoic   0.2579265  0.2977100  0.7996572
##
## $T2
## [1] 419.0686
##
## $F_stat
## [1] 45.71658
##
## $p_value
## [1] 0
```

```
hotellings_with_imputed_datasets(oliver4a_imputed, mu4a_not)
```

```
## $mean
##    palmitic palmitoleic     stearic       oleic    linoleic  eicosanoic
##  1229.74430   105.07950   273.90831  7358.31686   834.72222    42.36862
##   linolenic  eicosenoic
##    75.70923    37.74981
##
## $total_variance
##                 palmitic palmitoleic      stearic       oleic     linoleic
## palmitic      883.87051  211.027036  -85.2352493 -1512.53822   425.498769
## palmitoleic   211.02704   68.947001  -30.0982641  -389.36460   114.535864
## stearic       -85.23525  -30.098264   62.9809487   165.93912  -109.885630
## oleic       -1512.53822 -389.364602  165.9391193  3164.49394 -1207.797795
## linoleic      425.49877  114.535864 -109.8856297 -1207.79779   694.083510
## eicosanoic    -11.42616   -4.019373    5.1232173    17.76508    -7.751248
## linolenic      36.19891    9.342686   -6.5092673   -72.48437    26.394337
## eicosenoic    -13.80746   -2.412249    0.1821949    11.42777     1.533029
##              eicosanoic   linolenic  eicosenoic
## palmitic    -11.4261627  36.1989098 -13.8074565
## palmitoleic  -4.0193732   9.3426861  -2.4122485
## stearic       5.1232173  -6.5092673   0.1821949
## oleic        17.7650765 -72.4843703  11.4277733
## linoleic     -7.7512476  26.3943374   1.5330289
## eicosanoic    0.6661800  -0.3950576   0.2553076
## linolenic    -0.3950576   3.4957071   0.3250064
## eicosenoic    0.2553076   0.3250064   1.5677745
##
## $T2
## [1] 576.3325
##
## $F_stat
## [1] 57.63325
##
```

```
## $p_value
## [1] 0
```

Comparison between Regions 2 and 4

```r
cat("Hotelling's T^2 Statistic:", as.numeric(t_squared), "\n") # 189.7534
```

```
## Hotelling’s T^2 Statistic: 189.7534
```

```r
cat("F-statistic:", as.numeric(f_statistic), "\n") # 21.87435
```

```
## F-statistic: 21.87435
```

```r
cat("Numerator degrees of freedom:", df1, "\n")
```

```
## Numerator degrees of freedom: 2
```

```r
cat("Denominator degrees of freedom:", df2, "\n")
```

```
## Denominator degrees of freedom: 83
```

```r
cat("P-value:", as.numeric(p_value), "\n")
```

```
## P-value: 0
```

Assessment of Linoleic and Linolenic Acids

```r
paste("F-stat for region effect:", Fs)
```

```
## [1] "F-stat for region effect: 28.2676278919721"
```

```r
paste("df1:", df1, "df2:", df2)
```

```
## [1] "df1: 2 df2: 83"
```

```
paste("P-value:", 1-pf(Fs, df1, df2))
```

```
## [1] "P-value: 4.33867164417734e-10"
```