Capstone Project - Predicting Depression from plasma measurements

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Data preparation

First we load the data sheet downloaded from the Metabolomics website: https://www.metabolomicsworkbench.org/data/DRCCMetadata.php?Mode=Study&StudyID=ST000062&StudyType=MS&ResultType=1 I have edited the data set; some of the measured molecules have been removed from the list for simplicity and also due to these did not have names, only ID numbers. A simplified data sheet is used to do the analysis.

```
library("readxl")
library("dplyr")
library("ggplot2")
library('ggfortify')
library('corrplot')
library('stats')
library('purrr')
library('caTools')
library('e1071')
library('randomForest')
library('stringr')
library('class')
# Here, I set the file to a path on my computer, but once this is saved to a
# different computer, the path will need to be updated. The simplified data sheet
# can also be downloaded from my git repository:
# https://github.com/zsk2021/CapstoneProject--PredictingDepression
file_original <- "~/Desktop/HarvardX, EdX, Data Science/Capstone Project/Capstone Project - Chosen Proj
temp_file <- read_excel(file_original)
# Converting the temp file into a transposed data frame.
file_df <- as.data.frame(t(temp_file))
# removing unnecessary rows/columns
data <- file_df[c(-2,-3,-4),c(-1,-3,-4,-5,-7)]
```

New column names are introduced:

```
# Adding new column names for the molecules and the arm:
data[1,1] <- 'Samples'
data[1,2] <- 'Arm'
colnames(data) <-data[1,]
# Remove first row from the data frame:
data <- data[-1,]
# By investigating the data, we can see that measurements come from two groups:
# Group 1 (control) and Group 2 (patients diagnosed with depression):
data %>% group_by(Arm) %>% summarise(n = n())
```

```
## # A tibble: 2 x 2
##   Arm                     n
```

```
##   <chr>               <int>
## 1 Group 1 - Score 0     48
## 2 Group 2 - Score 50    49
```

To analyse the relationship between the different measurements between the two groups, I first separate the two arms and remove unnecessary columns:

```
group_1 <- data %>% group_by(Arm) %>% filter(Arm == "Group 1 - Score 0")
group_1_truncated <- group_1[, c(-1, -2)]
group_2 <- data %>% group_by(Arm) %>% filter(Arm == "Group 2 - Score 50")
group_2_truncated <- group_2[, c(-1, -2)]
```

Data analysis

Data distribution

I first look at the distribution of the data for each measurement for the two arms, by generating graphs with the codes below. Due to these produce many plots (144 plots per arm), I commented these out in the .rmd file.

```
# Group 1:

#for (i in group_1_truncated){
# plot <- group_1_truncated %>% ggplot(aes(x = as.numeric(i))) +
#  geom_density()
# print(plot)
#}

# Group 2

#for (i in group_2_truncated){
#  plot <- group_2_truncated %>% ggplot(aes(x = as.numeric(i))) +
#    geom_density()
#  print(plot)
#}
```

Instead, I used Shapiro-Wilk's method (http://www.sthda.com/english/wiki/normality-test-in-r) to get a value of the normality for each measured parameter. The null hypothesis for this test is that "the sample distribution is normal". So, if the p-value is >0.05, that implies that the distribution of the data is not significantly different from the normal distribution. In other words, if the p-value is >0.05 we can assume normality. First, I loop through the truncated and transposed list and generate Shapiro-Wilk's test for each column in the data set. I use the magicfor library to record p-values in a vector:

```
library(magicfor)
# Group 1:
magic_for(print)
for (c in group_1_truncated){
  # shap test for each col
  shap_test <- shapiro.test(as.numeric(c))
  output <- shap_test$p.value
  print(output)
```

```
}
# Saving the printed p-values as a vector:
pvalues_group_1 <- magic_result_as_vector()
# Binding vector to the original data, so the last row is the p-value from the
# Shapiro-Wilk's test:
group_1_truncated_with_pvalues <- rbind(group_1_truncated, pvalues_group_1)

# Group 2:
magic_for(print)
for (c in group_2_truncated){
  # shap test for each col
  shap_test <- shapiro.test(as.numeric(c))
  output <- shap_test$p.value
  print(output)
}
# Saving the printed p-values as a vector:
pvalues_group_2 <- magic_result_as_vector()
# Binding vector to the original data, so the last row is the p-value from the
# Shapiro-Wilk's test:
group_2_truncated_with_pvalues <- rbind(group_2_truncated,pvalues_group_2)
# Remove magicalization:
magic_free()
```

The last row in these two data frames are the Shapiro-Wilk's p-values:

```
group_1_truncated_with_pvalues %>%
  summarise(Arm = 'Group 2',
            nrow = dim(group_1_truncated_with_pvalues)[1],
            ncol = dim(group_1_truncated_with_pvalues)[2])
```

```
## # A tibble: 1 x 3
##   Arm      nrow  ncol
##   <chr>   <int> <int>
## 1 Group 2    49   143
```

```
group_2_truncated_with_pvalues %>%
  summarise(Arm = 'Group 2',
            nrow = dim(group_2_truncated_with_pvalues)[1],
            ncol = dim(group_2_truncated_with_pvalues)[2])
```

```
## # A tibble: 1 x 3
##   Arm      nrow  ncol
##   <chr>   <int> <int>
## 1 Group 2    50   143
```

Now, that I have the p-values for the Shapiro-Wilk's test for the measured parameters from each arm, I transpose the data frames, so the p-values are in a separate column and the data frame is in tidy format:

```
# Group 1 - transpose
group_1_tidy <- as.data.frame(t(group_1_truncated_with_pvalues))
# adding on extra row that will be used for the column names
```

```r
group_1_tidy<- add_row(group_1_tidy, .before = 1)
# using sample names for column names
group_1_tidy[1,1:48] <- group_1$Samples
# adding name for extra column
group_1_tidy[1,49] <- "Shapiro-Wilk's p-values"
# use first row as column names
colnames(group_1_tidy) <- group_1_tidy[1,]
group_1_tidy <- group_1_tidy[-1,]
# The last column is the Shapiro-Wilk's p-values
group_1_tidy %>% summarise(Arm = 'Group 1', nrow = dim(group_1_tidy)[1],
                           ncol = dim(group_1_tidy)[2])
```

```
##       Arm nrow ncol
## 1 Group 1  143   49
```

```r
# Group 2 -transpose
group_2_tidy <- as.data.frame(t(group_2_truncated_with_pvalues))
# adding on extra row that will be used for the column names
group_2_tidy<- add_row(group_2_tidy, .before = 1)
# using sample names for column names
group_2_tidy[1,1:49] <- group_2$Samples
# adding name for extra column
group_2_tidy[1,50] <- "Shapiro-Wilk's p-values"
# use first row as column names
colnames(group_2_tidy) <- group_2_tidy[1,]
group_2_tidy <- group_2_tidy[-1,]
# The last column is the Shapiro-Wilk's p-values
group_2_tidy %>% summarise(Arm = 'Group 2', nrow = dim(group_2_tidy)[1],
                           ncol = dim(group_2_tidy)[2])
```

```
##       Arm nrow ncol
## 1 Group 2  143   50
```

Now, that I have the data for the two arms, together with the p-values for normal distribution, I filter the data to keep the measured parameters, where the distribution was approximately normal. In other words, I keep all measured parameters, where the p-value was >0,05:

```r
group_1_tidy <- group_1_tidy %>% filter(`Shapiro-Wilk's p-values`>0.05)
group_2_tidy <- group_2_tidy %>% filter(`Shapiro-Wilk's p-values`>0.05)
# There are 97 and 112 measured parameters where the p-value is >0.05 in Group 1
# and Group 2, respectively. Group 1 has 48 patients, whereas Group 2 has 49. The extra
# column in each data frame is the Shapiro-Wilk's p-value.
group_1_tidy %>% summarise(Arm = 'Group 1', nrow = dim(group_1_tidy)[1],
                           ncol = dim(group_1_tidy)[2])
```

```
##       Arm nrow ncol
## 1 Group 1   97   49
```

```r
group_2_tidy %>% summarise(Arm = 'Group 2', nrow = dim(group_2_tidy)[1],
                           ncol = dim(group_2_tidy)[2])
```

```
##        Arm nrow ncol
## 1 Group 2  112   50
```

Due to the number of the normally distributed measured parameters are different in the two groups, I will work with the list from the control group (Group 1 - baseline), where the normally distributed parameters were 97 (as opposed to Group 2 where it was 112). I use semi_join to keep only the records from Group 2, that have a match in Group 1.

```
# Adding row names as an extra column, so I can use semi_join:
group_1_tidy <- cbind(group_1_tidy, rownames = rownames(group_1_tidy))
group_2_tidy <- cbind(group_2_tidy, rownames = rownames(group_2_tidy))
# we should have one extra column in each data frame:
group_1_tidy %>% summarise(Arm = 'Group 1', nrow = dim(group_1_tidy)[1],
                           ncol = dim(group_1_tidy)[2])
```

```
##        Arm nrow ncol
## 1 Group 1   97   50
```

```
group_2_tidy %>% summarise(Arm = 'Group 2', nrow = dim(group_2_tidy)[1],
                           ncol = dim(group_2_tidy)[2])
```

```
##        Arm nrow ncol
## 1 Group 2  112   51
```

```
# Keep everything from Group 1 with a match in Group 2:
group_1_tidy <- semi_join(group_1_tidy, group_2_tidy, by = "rownames")
# Keep everything from Group 2 with a match in Group 1:
group_2_tidy <- semi_join(group_2_tidy, group_1_tidy, by = "rownames")
# Investigating the dimensions of the two newly generated data frames, we can see, that
# both arms have 78 measured parameters, as well as 48 and 49 sample count (plus the two columns
# with p-values and row names), respectively.
group_1_tidy %>% summarise(Arm = 'Group 1', nrow = dim(group_1_tidy)[1],
                           ncol = dim(group_1_tidy)[2])
```

```
##        Arm nrow ncol
## 1 Group 1   78   50
```

```
group_2_tidy %>% summarise(Arm = 'Group 2', nrow = dim(group_2_tidy)[1],
                           ncol = dim(group_2_tidy)[2])
```

```
##        Arm nrow ncol
## 1 Group 2   78   51
```

Two-sample t-test

In this next section, I will calculate two-sample t-tests for the selected parameters, so I can see if there is a significant difference in any parameters between the two groups. First, I transpose the data frame generated above and remove unnecessary rows.

```r
# Transpose tidy data, so I can loop through the columns: Group 1
group_1_tidy_t <- as.data.frame(t(group_1_tidy))
# Removing last two rows with p-values and row names:
group_1_tidy_t <- group_1_tidy_t[c(-49,-50),]
# Transpose tidy data, so I can loop through the columns: Group 2
group_2_tidy_t <- as.data.frame(t(group_2_tidy))
# Removing last two rows with p-values and row names:
group_2_tidy_t <- group_2_tidy_t[c(-50,-51),]
# The two data set has 78 measured parameters and 48 and 49 samples, respectively:
group_1_tidy_t %>% summarise(Arm = 'Group 1', nrow = dim(group_1_tidy_t)[1],
                             ncol = dim(group_1_tidy_t)[2])
```

```
##       Arm nrow ncol
## 1 Group 1   48   78
```

```r
group_2_tidy_t %>% summarise(Arm = 'Group 2', nrow = dim(group_2_tidy_t)[1],
                             ncol = dim(group_2_tidy_t)[2])
```

```
##       Arm nrow ncol
## 1 Group 2   49   78
```

Now, that I have the two data frames with the same measured parameters in both, and all of the measurements show approximately normal distribution, I can test the vectors for significant differences:

```r
# Two-sample t-test by looping through the columns:
magic_for(print)
for (j in seq(ncol(group_1_tidy_t))){
  testresults <- t.test(as.numeric(group_1_tidy_t[,j]), as.numeric(group_2_tidy_t[,j]))
  print(testresults$p.value)
}
```

Saving p-values from the two-sample t-test into a data frame:

```r
twosample_ttest <- magic_result_as_dataframe()
magic_free()
# Adding the names of the measured parameters to the p-values:
colnames(twosample_ttest)[1] <- 'rownames'
twosample_ttest$`rownames` <- colnames(group_1_tidy_t)
# Filtering out measured parameters that showed significant differences between
# the two groups:
twosample_ttest_significant <- twosample_ttest %>% filter(`testresults$p.value` <= 0.05)
# There are 15 measured parameters that show normal distribution, and there is a significant difference
# between the two groups:
twosample_ttest_significant
```

```
##              rownames testresults$p.value
## 1        stearic acid        4.942357e-05
## 2            sorbitol        4.571605e-02
## 3       shikimic acid        1.102079e-02
## 4             ribitol        4.199180e-02
## 5      pseudo uridine        1.947676e-03
```

```
## 6         nicotinamide        5.894853e-04
## 7         myo-inositol        1.060330e-03
## 8              mannose        2.861927e-02
## 9              lyxitol        2.000258e-03
## 10  heptadecanoic acid        1.549329e-04
## 11        glutaric acid        4.067713e-02
## 12        glutamic acid        1.122825e-02
## 13          citric acid        2.823496e-02
## 14         behenic acid        9.529080e-04
## 15 alpha-ketoglutarate        1.629588e-03
```

Correlation analysis

From the previous section, I have a set of measured parameters that show significant difference of the mean between the two arms. To see the actual relationship between the two groups, I will use correlation analysis for the 15 parameters. Initially, I will subset the two data frames group_1_tidy and group_2_tidy to only consist of the 15 parameters of interest.

```
group_1_final <- semi_join(group_1_tidy, twosample_ttest_significant, by = 'rownames')
group_1_final <- group_1_final[,c(-49,-50)]

group_2_final <- semi_join(group_2_tidy, twosample_ttest_significant, by = 'rownames')
group_2_final <- group_2_final[,c(-50,-51)]

# Here I have two data frames from the two arms, one control and one diagnosed with depression,
# where only the parameters of interest are included The data frames consist of 48 and 49
# patients, respectively:
group_1_final %>% summarise(Arm = 'Group 1', nrow = dim(group_1_final)[1],
                            ncol = dim(group_1_final)[2])
```

```
##       Arm nrow ncol
## 1 Group 1   15   48
```

```
group_2_final %>% summarise(Arm = 'Group 2', nrow = dim(group_2_final)[1],
                            ncol = dim(group_2_final)[2])
```

```
##       Arm nrow ncol
## 1 Group 2   15   49
```

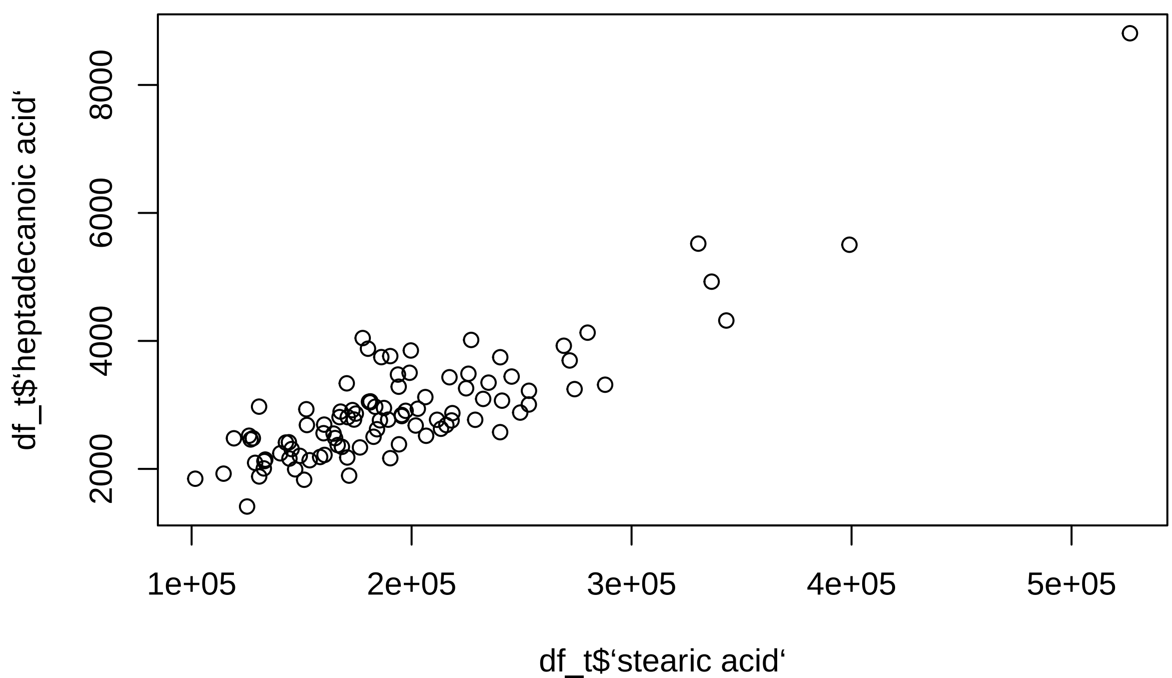
Now that I have the two data frames with the 15 measured parameters that showed approximately normal distribution and significant differences between the two groups, I will merge the two arms, and will generate a new data frame with all of the subjects and the 15 measured parameters. I will use this data frame for my further work:

```
# First, I create a new column in both data frames, so I can merge these with
# left_join()
group_1_final <- cbind(group_1_final, rownames = rownames(group_1_final))
group_2_final <- cbind(group_2_final, rownames = rownames(group_2_final))
# Merging the two data frames by rownames:
df <- left_join(group_1_final, group_2_final, by = "rownames")
# Adding rownames based on the rownames column
```

```
rownames(df) <- df$rownames
# Removing rownames column results in the final data set df:
df <- subset(df, select = -rownames)
```

Testing for correlation:

```
# In the above section, I generated my data set with all 15 parameters and the entire cohort.
# I now transpose this and will do a correlation analysis to see if any of these
# parameters are correlated:
df_t <- as.data.frame(t(df))
# A quick plotting of the data shows that there is a potential correlation between
# stearic acid and heptadecanoic acid: commented out code, otherwise too many plots
# would have been printed.
# plot(df_t)
plot(df_t$`stearic acid`, df_t$`heptadecanoic acid`)
```




```
# A correlation analysis between the two parameters shows a strong positive correlation
# with a value of 0.862:
cor.test(as.numeric(df_t$`stearic acid`), as.numeric(df_t$`heptadecanoic acid`))
```

```
##
##  Pearson's product-moment correlation
##
## data:  as.numeric(df_t$'stearic acid') and as.numeric(df_t$'heptadecanoic acid')
```

```
## t = 16.583, df = 95, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.8002618 0.9058056
## sample estimates:
##       cor
## 0.8621075
```

```
# Here, I convert all df_t to numeric, so I can do a correlation analysis:
df_num <-as.data.frame(sapply(df_t, as.numeric))
# This also shows, that the only correlation is between stearic acid and heptadecanoic acid:
cor_15_param <- as.data.frame(cor(df_num))
cor_15_param %>% filter(cor_15_param >= 0.7)
```

```
##                    stearic acid  sorbitol shikimic acid    ribitol
## stearic acid          1.0000000 0.2083188     0.2492042 0.15060595
## heptadecanoic acid    0.8621075 0.1501909     0.3128487 0.09976116
##                    pseudo uridine nicotinamide myo-inositol   mannose
## stearic acid          -0.19706050    0.3878188   0.04917488 0.1807085
## heptadecanoic acid    -0.05231672    0.2501691   0.13140503 0.2167668
##                       lyxitol heptadecanoic acid glutaric acid glutamic acid
## stearic acid       0.02011295          0.8621075  -0.034593242    0.05591120
## heptadecanoic acid 0.01543889          1.0000000  -0.005699254   -0.04747537
##                    citric acid behenic acid alpha-ketoglutarate
## stearic acid        0.08475043    0.4184708          0.02048958
## heptadecanoic acid  0.17991391    0.3845567         -0.01077332
```

Linearity between the two arms

I have also looked at the other features, whether these are linearly separable between the two arms. Values from each measured parameters are plotted on y and the arm is plotted on x.

```
# code is commented to avoid the printing of an excess amount of graphs:
# for (v in data[,c(-1,-2)]){
#  plot(as.numeric(v), col = as.factor(data$Arm))
# }
```

Based on the plots generated by the above code, the values do not show a linear association between the two arms.

Model fitting

Creating the train and test data sets

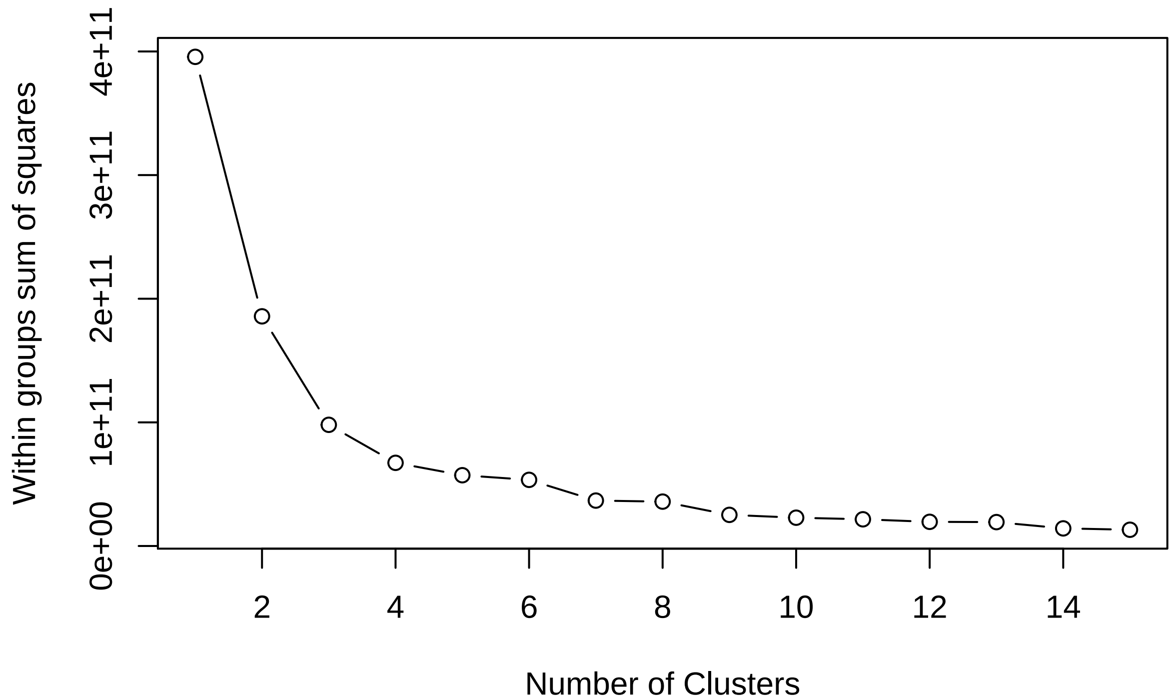
```
# Creating a training and test set from the data frame df_num:
# First I add the arm to the data set, so the split can be done based on these features:
df_num_sp <- cbind(df_num, Arm = data$Arm)
index <- sample.split(df_num_sp$Arm, SplitRatio = .7)
tr_set <- subset(df_num_sp, index == TRUE)
```

```
final_val_set <- subset(df_num_sp, index == FALSE)
# Splitting the training set into further training and test sets:
index_train <- sample.split(tr_set$Arm, SplitRatio = .5)
tr_set_train <- subset(tr_set, index_train == TRUE)
tr_set_test <- subset(tr_set, index_train == FALSE)
```

K-means Clustering

I used K-means clustering to see the structure of the data, however, the number of data points in each groups did not agree with the actual sample numbers in each arms:

```
# I used the df_t data set, that is a transposed format of the 15 significant measurements
# from the two arms. Parameters are in the columns, and samples in rows. Here we can see
# the group sizes and the ratio of the between sum of squares to the total sum of
# squares. For this latter ratio, the high number would suggest a good fit for the clustering
# scheme to the data.
# First, lets find the optimum number of clusters: we use the wssplot() function,
# of which the code was copied from this website: https://www.projectpro.io/data-science-in-r-programmi
wssplot <- function(data, nc=15, seed=1234){
                  wss <- (nrow(data)-1)*sum(apply(data,2,var))
                      for (i in 2:nc){
                set.seed(seed)
                    wss[i] <- sum(kmeans(data, centers=i)$withinss)}
              plot(1:nc, wss, type="b", xlab="Number of Clusters",
                            ylab="Within groups sum of squares")
              wss
       }
wssplot(df_num)
```



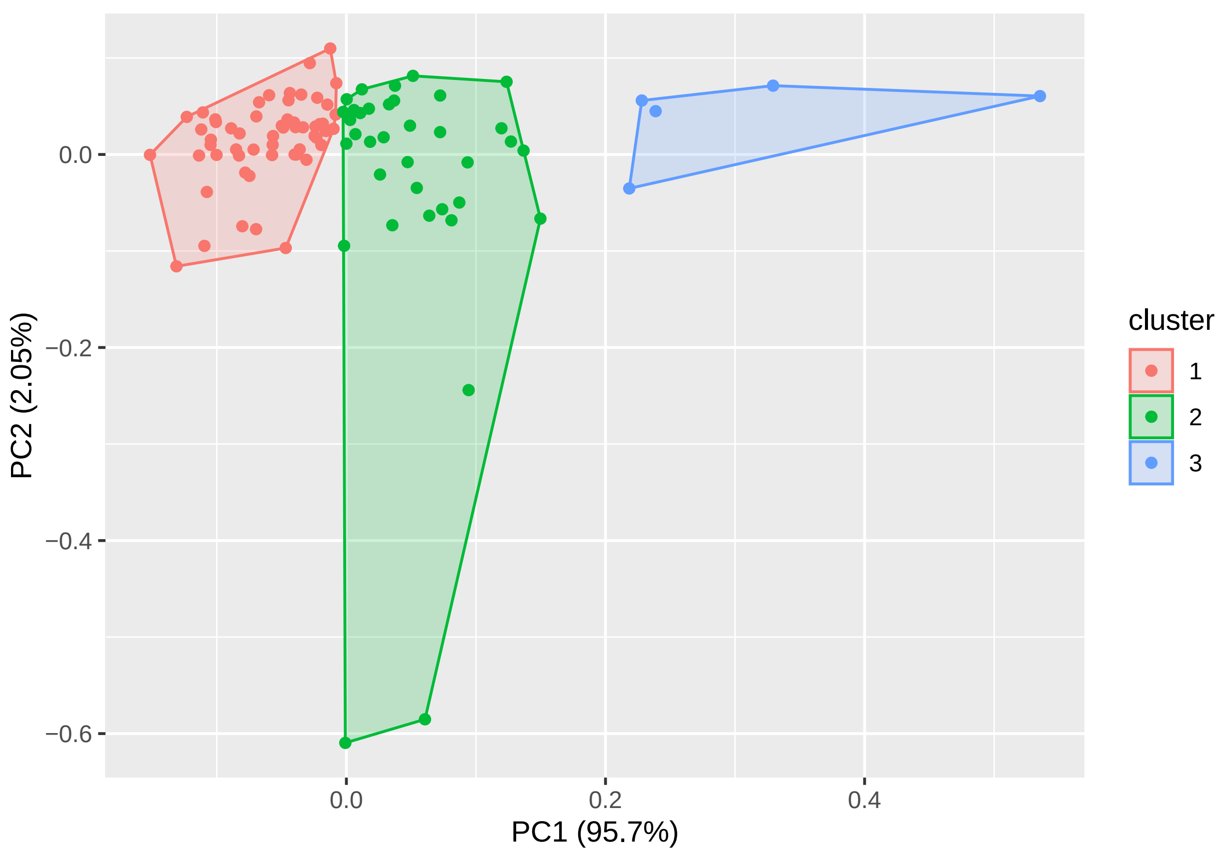

```
##  [1] 395635828037 185760569486  98013679493  67263552575  57250341995
##  [6]  53490348440  36742712995  35905094218  25183107256  22902610178
## [11]  21609713450  19544771548  19333076704  14248401371  13151570164
```

```r
# From the above plot, we can see that the optimum number of clusters 3 (the smallest
# possible number, where the plot shows an elbow shape). So, we will apply the cluster numbers 3
# in our k-means cluster analysis.
KM_3 <- kmeans(df_num, 3)
print(KM_3)
```

```
## K-means clustering with 3 clusters of sizes 56, 36, 5
##
## Cluster means:
##   stearic acid  sorbitol shikimic acid  ribitol pseudo uridine nicotinamide
## 1     158133.1  984.8479      376.7243 342.5996       1425.287     159.2324
## 2     226722.3 3758.6777      404.5479 566.9193       1330.800     428.6950
## 3     387081.3 4226.8173      616.2581 434.8046       1148.915     944.4025
##   myo-inositol  mannose  lyxitol heptadecanoic acid glutaric acid glutamic acid
## 1     8138.416 15893.98 1085.131           2557.490      90.20281      4888.893
## 2     8756.183 14255.69 1170.427           3158.250      85.15203      9104.517
## 3     7566.377 19499.29 1016.876           5815.383      77.25767      4250.937
##   citric acid behenic acid alpha-ketoglutarate
## 1    29348.89     592.8050            175.9726
## 2    29345.78     758.3372            178.3793
## 3    29944.92     927.8507            216.9687
```

```
##
## Clustering vector:
##  [1] 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 2 1 1 1 1 2 1 1 1 1 1 1 1 1 2 1 1 1 2 2 1 2
## [39] 1 1 2 2 2 1 2 1 1 1 2 3 1 2 1 2 2 2 2 2 2 2 1 2 2 2 2 3 2 2 1 2 2 1 1 2 2 1
## [77] 2 2 3 3 2 1 3 1 1 1 1 1 1 1 1 2 1 1 2 2 1
##
## Within cluster sum of squares by cluster:
## [1] 33485743443 36586389829 27941546221
##  (between_SS / total_SS =  75.2 %)
##
## Available components:
##
## [1] "cluster"      "centers"      "totss"        "withinss"     "tot.withinss"
## [6] "betweenss"    "size"         "iter"         "ifault"
```

```r
# Visualizing the two clusters, to see whether these are distinct enough , or not.
# There are two ways to evaluate cluster analysis: 1.) looking at the cluster plot or
# or 2.) look at the cluster centers.
# Fist we look at the cluster plot by using the autoplot() function:
autoplot(KM_3, df_num, frame = TRUE)
```

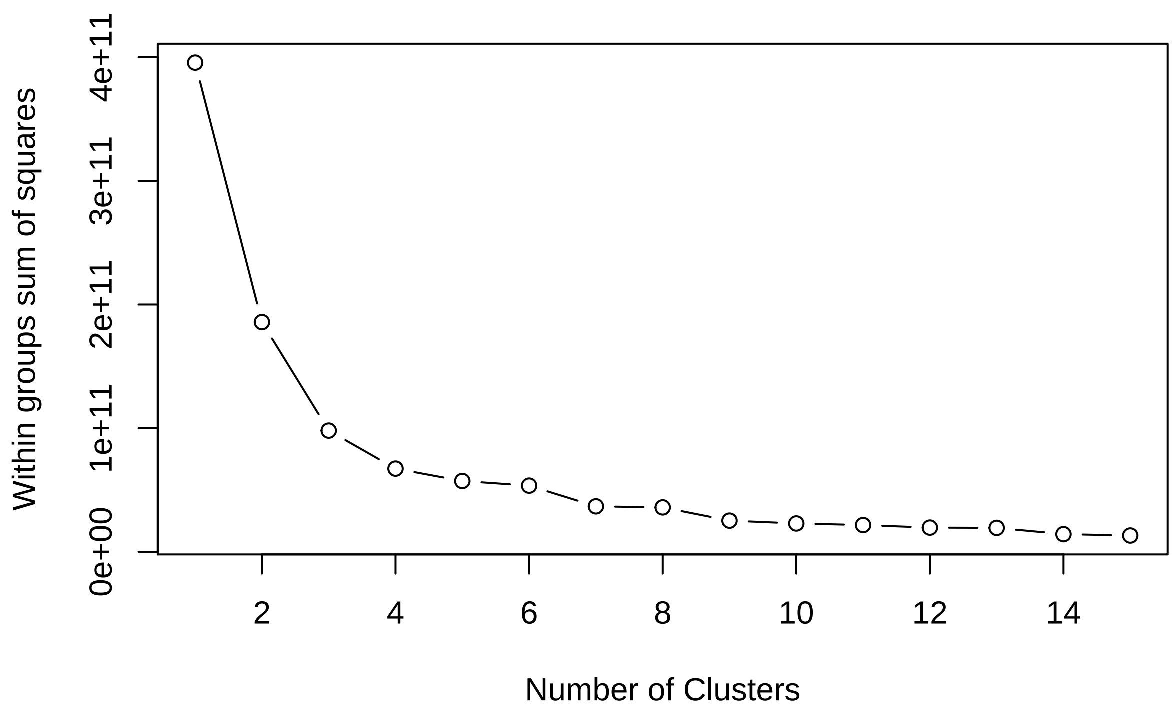



```r
# From the above plot, we can see that the clusters 1 and 2 overlap and there is no clear
# separation between the classes. As the number of observation increases, the cluster plot becomes more
# 'busy', therefore, another way to evaluate the k-means cluster analysis and see the distinctiveness
# of the clusters is to look at the center of the particular clusters. Centroids
```

```r
# can be derived from the k-means analysis object:
KM_3$centers
```

```
##   stearic acid  sorbitol shikimic acid  ribitol pseudo uridine nicotinamide
## 1     158133.1  984.8479      376.7243 342.5996       1425.287     159.2324
## 2     226722.3 3758.6777      404.5479 566.9193       1330.800     428.6950
## 3     387081.3 4226.8173      616.2581 434.8046       1148.915     944.4025
##   myo-inositol  mannose  lyxitol heptadecanoic acid glutaric acid glutamic acid
## 1     8138.416 15893.98 1085.131           2557.490      90.20281      4888.893
## 2     8756.183 14255.69 1170.427           3158.250      85.15203      9104.517
## 3     7566.377 19499.29 1016.876           5815.383      77.25767      4250.937
##   citric acid behenic acid alpha-ketoglutarate
## 1    29348.89     592.8050            175.9726
## 2    29345.78     758.3372            178.3793
## 3    29944.92     927.8507            216.9687
```

```r
# # From the above values, we can see that the centers of the selected parameters are
# different, suggesting that the clusters are distinct in nature. A good separation for
# the clusters in the case of stearic acid, nicotinamide, myo-inositol, mannose,
# heptadecanoic acid, and glutamic acid can be seen.
# Now, if I repeat the same analysis with only two clusters (based on the 2 arms and
# also on a potential elbow on the below plot at cluster 2), I can see a better separation
# for the centers in the case of stearic acid, sorbitol, nicotinamide, heptadecanoic acid,
# glutamic acid, and behenic acid.
wssplot(df_num)
```

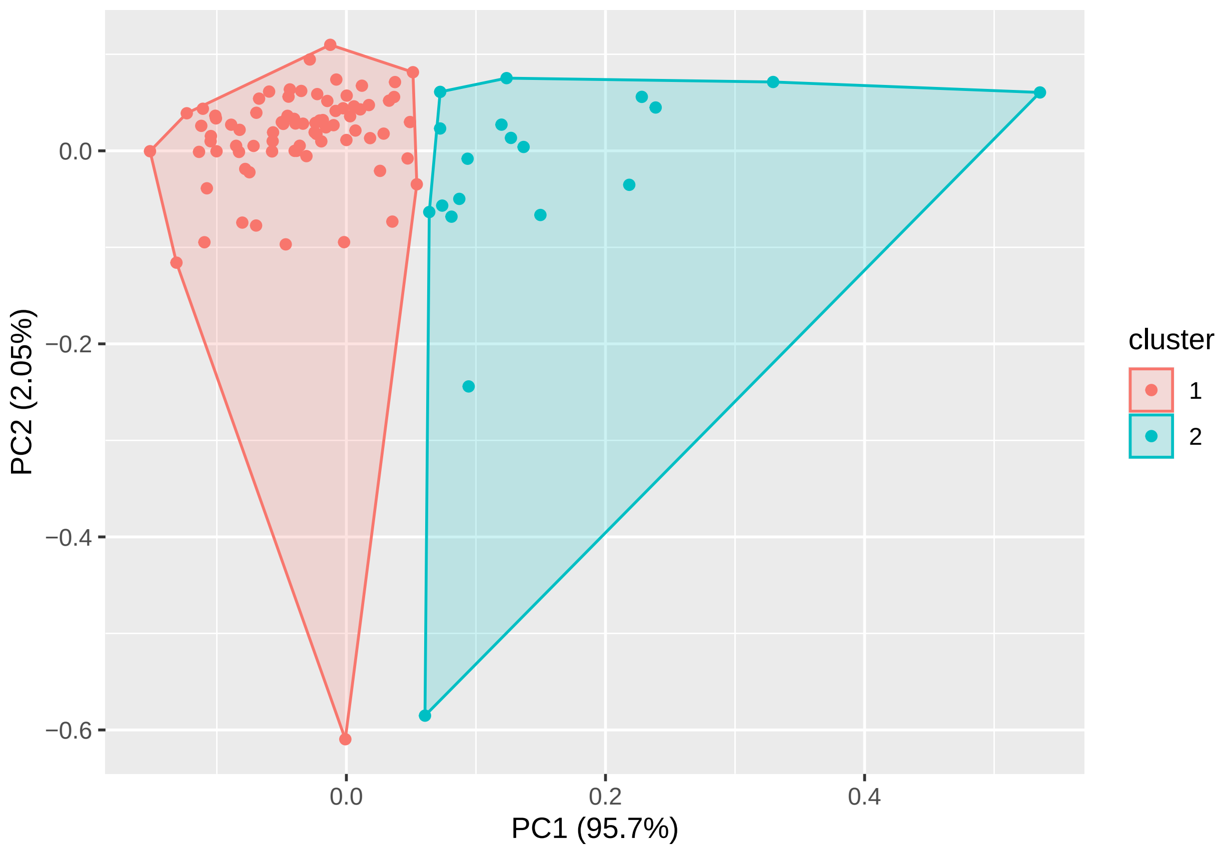


```
##  [1] 395635828037 185760569486  98013679493  67263552575  57250341995
##  [6]  53490348440  36742712995  35905094218  25183107256  22902610178
## [11]  21609713450  19544771548  19333076704  14248401371  13151570164
```

```r
KM_2 <- kmeans(df_num, 2)
print(KM_2)
```

```
## K-means clustering with 2 clusters of sizes 78, 19
##
## Cluster means:
##   stearic acid sorbitol shikimic acid  ribitol pseudo uridine nicotinamide
## 1     172353.6 1680.032      382.2930 403.4542       1394.712     217.5227
## 2     289962.4 4239.760      469.6168 542.0666       1299.050     637.1195
##   myo-inositol  mannose  lyxitol heptadecanoic acid glutaric acid glutamic acid
## 1     8367.910 15398.69 1105.755           2695.191      88.42112      5481.488
## 2     8216.252 15771.91 1144.115           3987.812      84.54062     10275.749
##   citric acid behenic acid alpha-ketoglutarate
## 1    29588.69     630.8289            175.5881
## 2    28515.39     838.5168            192.8996
##
## Clustering vector:
##  [1] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2
## [39] 1 1 2 1 1 1 1 1 1 1 1 2 1 2 1 1 1 1 2 2 2 1 1 2 2 1 2 2 2 1 1 1 1 1 1 2 2 1
## [77] 2 2 2 2 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1
##
## Within cluster sum of squares by cluster:
## [1]  82453715161 101353923427
##  (between_SS / total_SS =  53.5 %)
##
## Available components:
##
## [1] "cluster"      "centers"      "totss"        "withinss"     "tot.withinss"
## [6] "betweenss"    "size"         "iter"         "ifault"
```

```r
autoplot(KM_2, df_num, frame = TRUE)
```




```
KM_2$centers
```

```
##   stearic acid sorbitol shikimic acid  ribitol pseudo uridine nicotinamide
## 1     172353.6 1680.032      382.2930 403.4542       1394.712     217.5227
## 2     289962.4 4239.760      469.6168 542.0666       1299.050     637.1195
##   myo-inositol  mannose  lyxitol heptadecanoic acid glutaric acid glutamic acid
## 1     8367.910 15398.69 1105.755           2695.191      88.42112      5481.488
## 2     8216.252 15771.91 1144.115           3987.812      84.54062     10275.749
##   citric acid behenic acid alpha-ketoglutarate
## 1    29588.69     630.8289            175.5881
## 2    28515.39     838.5168            192.8996
```

However, if we compare the within cluster sum of squares for the first and the second run (3 and 2 clusters, respectively), we can see that the analysis with 2 clusters is a less good fit (53.5%) than that of the 3 clusters (75.2%). Hence, I will be using the parameters selected from the k-means analysis ran with 3 clusters.

Support Vector Machine

Due to I am predicting a categorical variable, using numeric variables, this is a classical example of a classification model. In this example, the arm is the predicted, whereas the parameters are the predictor variables. Now, that I have the parameters that show the best separation,I will train the SVM on the training set. Here I train the SVM to predict the Arm, based on the variables that showed significant difference between the two arms, and gave the best cluster separation: stearic acid, sorbitol, nicotinamide, mannose, heptadecanoic acid, glutaric acid, glutamic acid and behenic acid.

```r
# SVM model with linear kernel
svm_model_linear <- svm(as.factor(Arm)~
                          as.numeric(tr_set_train$`stearic acid`) +
                          as.numeric(tr_set_train$sorbitol) +
                          as.numeric(tr_set_train$nicotinamide) +
                          as.numeric(tr_set_train$mannose) +
                          as.numeric(tr_set_train$`heptadecanoic acid`) +
                          as.numeric(tr_set_train$`glutaric acid`) +
                          as.numeric(tr_set_train$`glutamic acid`) +
                          as.numeric(tr_set_train$`behenic acid`),
                        data = tr_set_train, method = "C", kernel = "linear",
                        gamma = 1, cost = 2)
# Getting the mean of the correctly predicted arm on the train data set:
predict_tr_linear <- predict(svm_model_linear, tr_set_train)
mean_linear_train <- mean(predict_tr_linear == tr_set_train$Arm)
mean_linear_train
```

```
## [1] 0.8235294
```

```r
# Getting the mean of the correctly predicted arm on the test data set:
predict_test_linear <- predict(svm_model_linear, tr_set_test)
mean_linear_test <- mean(predict_test_linear == tr_set_test$Arm)
mean_linear_test
```

```
## [1] 0.8235294
```

```r
# SVM model with radial kernel:
svm_model_radial <- svm(as.factor(Arm)~
                          as.numeric(tr_set_train$`stearic acid`) +
                          as.numeric(tr_set_train$sorbitol) +
                          as.numeric(tr_set_train$nicotinamide) +
                          as.numeric(tr_set_train$mannose) +
                          as.numeric(tr_set_train$`heptadecanoic acid`) +
                          as.numeric(tr_set_train$`glutaric acid`) +
                          as.numeric(tr_set_train$`glutamic acid`) +
                          as.numeric(tr_set_train$`behenic acid`),
                 data = tr_set_train, method = "C-classification",
                 kernel = "radial", gamma = 1, cost = 1)
# Getting the mean of the correctly predicted arm on the train data set:
predict_tr_radial <- predict(svm_model_radial, tr_set_train)
mean_radial_train <- mean(predict_tr_radial == tr_set_train$Arm)
mean_radial_train
```

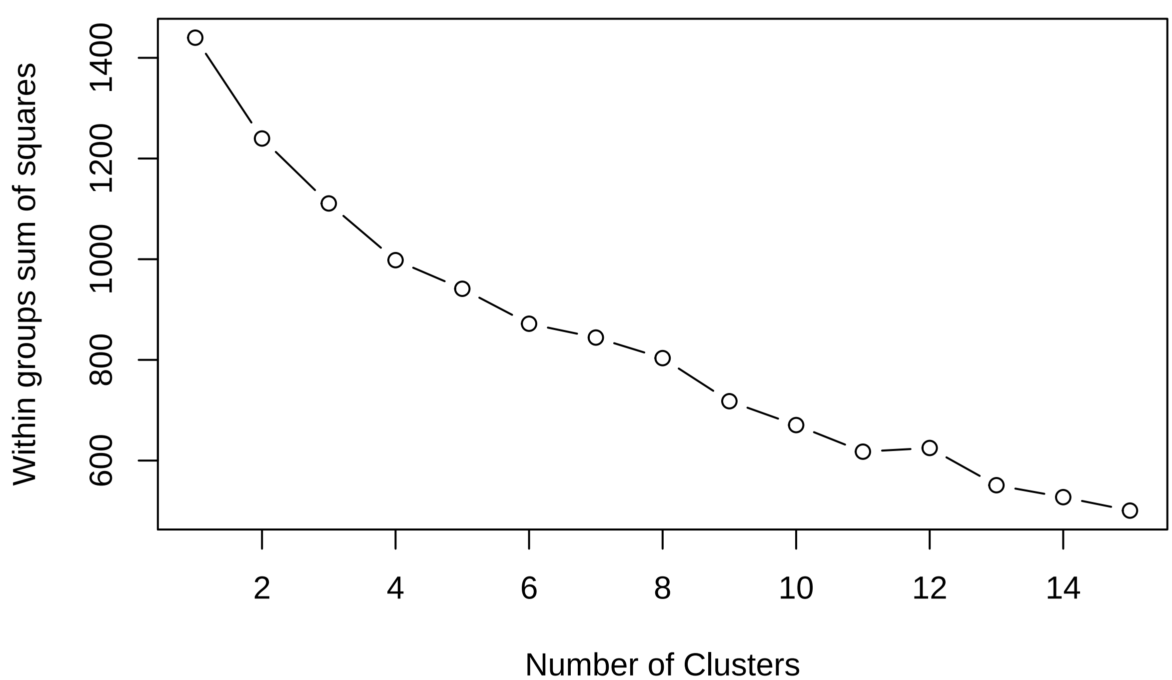
```
## [1] 1
```

```r
# Getting the mean of the correctly predicted arm on the test data set:
predict_test_radial <- predict(svm_model_radial, tr_set_test)
mean_radial_test <- mean(predict_test_radial == tr_set_test$Arm)
mean_radial_test
```

```
## [1] 1
```

Next, I will apply SVM on the standardized data set. I will use the scale() function, that calculates the Z-score for each variable.

```
# Creating a standardized training and test set from the data frame df_num:
df_num_st <- as_tibble(scale(df_num))
df_num_st <- cbind(df_num_st, Arm = data$Arm)
index_st <- sample.split(df_num_st$Arm, SplitRatio = .7)
tr_set_st <- subset(df_num_st, index == TRUE)
final_val_set_st <- subset(df_num_st, index == FALSE)
# Splitting the standardized training set into further training and test sets:
index_train_st <- sample.split(tr_set_st$Arm, SplitRatio = .5)
tr_set_train_st <- subset(tr_set_st, index_train_st == TRUE)
tr_set_test_st <- subset(tr_set_st, index_train_st == FALSE)
# Getting the wssplot to see the optimum number of clusters:
wssplot(df_num_st[,-16])
```



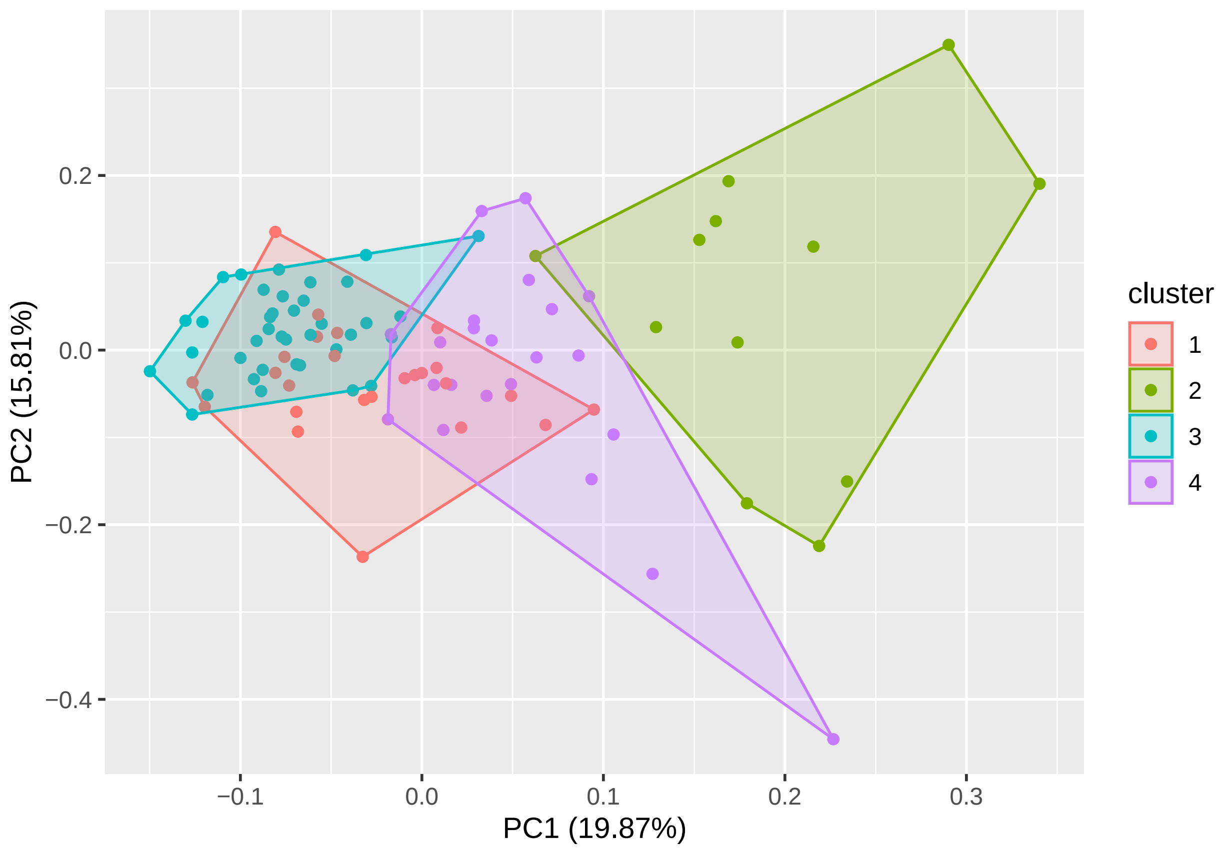

```
##  [1] 1440.0000 1239.7362 1110.7229  998.0776  941.2579  871.8389  844.3656
##  [8]  803.5112  717.8947  670.4786  617.6841  624.9956  551.0485  527.2994
## [15]  500.6391
```

```
KM_3_st <- kmeans(df_num_st[,-16], 4)
print(KM_3_st)
```

```
## K-means clustering with 4 clusters of sizes 25, 12, 38, 22
##
```

```
## Cluster means:
##   stearic acid   sorbitol shikimic acid     ribitol pseudo uridine nicotinamide
## 1   0.06844681 -0.2669806    -0.3202844 -0.15950256    -0.88662341  -0.03342757
## 2   0.73685272  1.7599490    -0.3472287  1.32721395     0.21080971   1.35636144
## 3  -0.60414659 -0.2635478    -0.1769679 -0.36062779     0.05579638  -0.36299136
## 4   0.56382580 -0.2013661     0.8590289  0.08022058     0.79616393  -0.07486257
##   myo-inositol    mannose    lyxitol heptadecanoic acid glutaric acid
## 1   -0.5286942 -0.9453649 -0.4658613        -0.04254934   -0.06128349
## 2    0.8935271  0.1667029  1.0893447         0.43558753   -0.75930630
## 3   -0.3174496  0.2630154 -0.2666614        -0.59320629    0.20208802
## 4    0.6617325  0.5290502  0.3957967         0.83538738    0.13474628
##   glutamic acid citric acid behenic acid alpha-ketoglutarate
## 1   -0.08845997  -0.2470422   0.46420620          0.26735648
## 2    1.09491648  -0.5222179   0.88853374          0.90100197
## 3   -0.22897933  -0.1666478  -0.57758347         -0.43341732
## 4   -0.10119473   0.8534220  -0.01451763         -0.04663989
##
## Clustering vector:
##  [1] 3 3 3 3 1 3 3 3 1 3 1 1 3 3 3 3 4 3 3 3 3 3 3 3 3 4 3 1 3 1 1 4 3 3 1 1 3 1
## [39] 3 3 1 1 2 3 1 1 3 3 2 4 1 1 1 1 4 1 4 1 1 3 3 2 2 2 4 2 1 4 2 4 1 4 4 2 4 4
## [77] 1 2 2 4 3 3 4 4 3 4 4 4 1 3 4 2 4 3 4 2 3
##
## Within cluster sum of squares by cluster:
## [1] 202.0444 328.9588 175.4584 341.5289
##  (between_SS / total_SS =  27.2 %)
##
## Available components:
##
## [1] "cluster"      "centers"      "totss"        "withinss"     "tot.withinss"
## [6] "betweenss"    "size"         "iter"         "ifault"
```

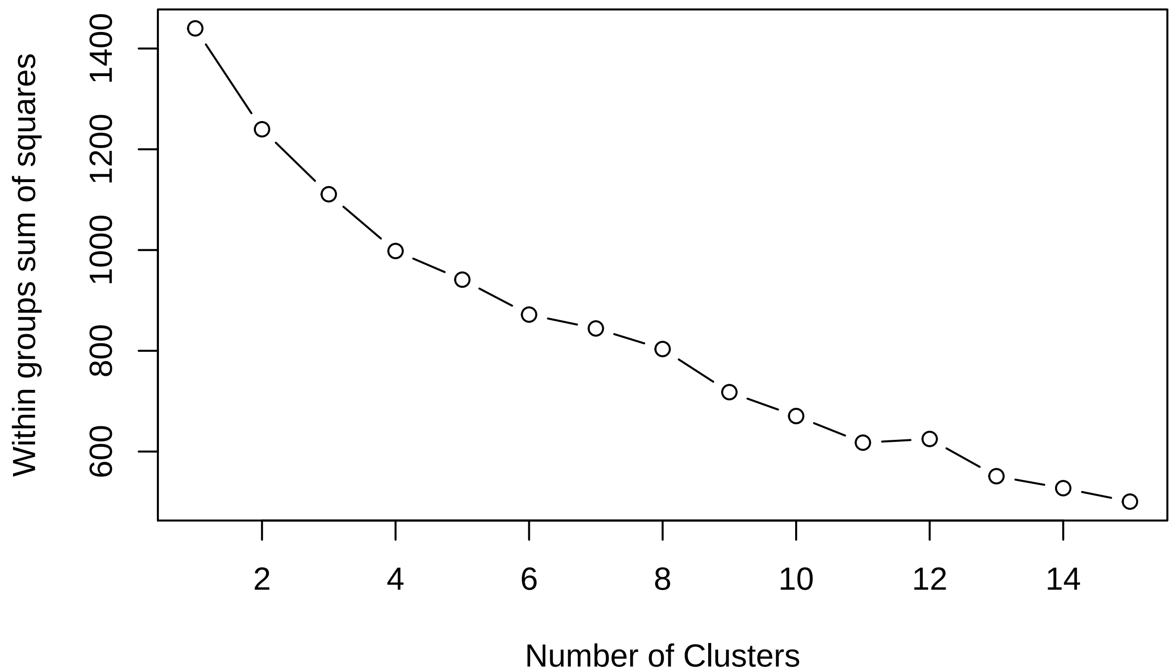
```
autoplot(KM_3_st, df_num_st[,-16], frame = TRUE)
```




```
KM_3_st$centers
```

```
##   stearic acid   sorbitol shikimic acid     ribitol pseudo uridine nicotinamide
## 1   0.06844681 -0.2669806    -0.3202844 -0.15950256    -0.88662341  -0.03342757
## 2   0.73685272  1.7599490    -0.3472287  1.32721395     0.21080971   1.35636144
## 3  -0.60414659 -0.2635478    -0.1769679 -0.36062779     0.05579638  -0.36299136
## 4   0.56382580 -0.2013661     0.8590289  0.08022058     0.79616393  -0.07486257
##   myo-inositol    mannose    lyxitol heptadecanoic acid glutaric acid
## 1   -0.5286942 -0.9453649 -0.4658613        -0.04254934   -0.06128349
## 2    0.8935271  0.1667029  1.0893447         0.43558753   -0.75930630
## 3   -0.3174496  0.2630154 -0.2666614        -0.59320629    0.20208802
## 4    0.6617325  0.5290502  0.3957967         0.83538738    0.13474628
##   glutamic acid citric acid behenic acid alpha-ketoglutarate
## 1   -0.08845997  -0.2470422   0.46420620          0.26735648
## 2    1.09491648  -0.5222179   0.88853374          0.90100197
## 3   -0.22897933  -0.1666478  -0.57758347         -0.43341732
## 4   -0.10119473   0.8534220  -0.01451763         -0.04663989
```

```
wssplot(df_num_st[,-16])
```



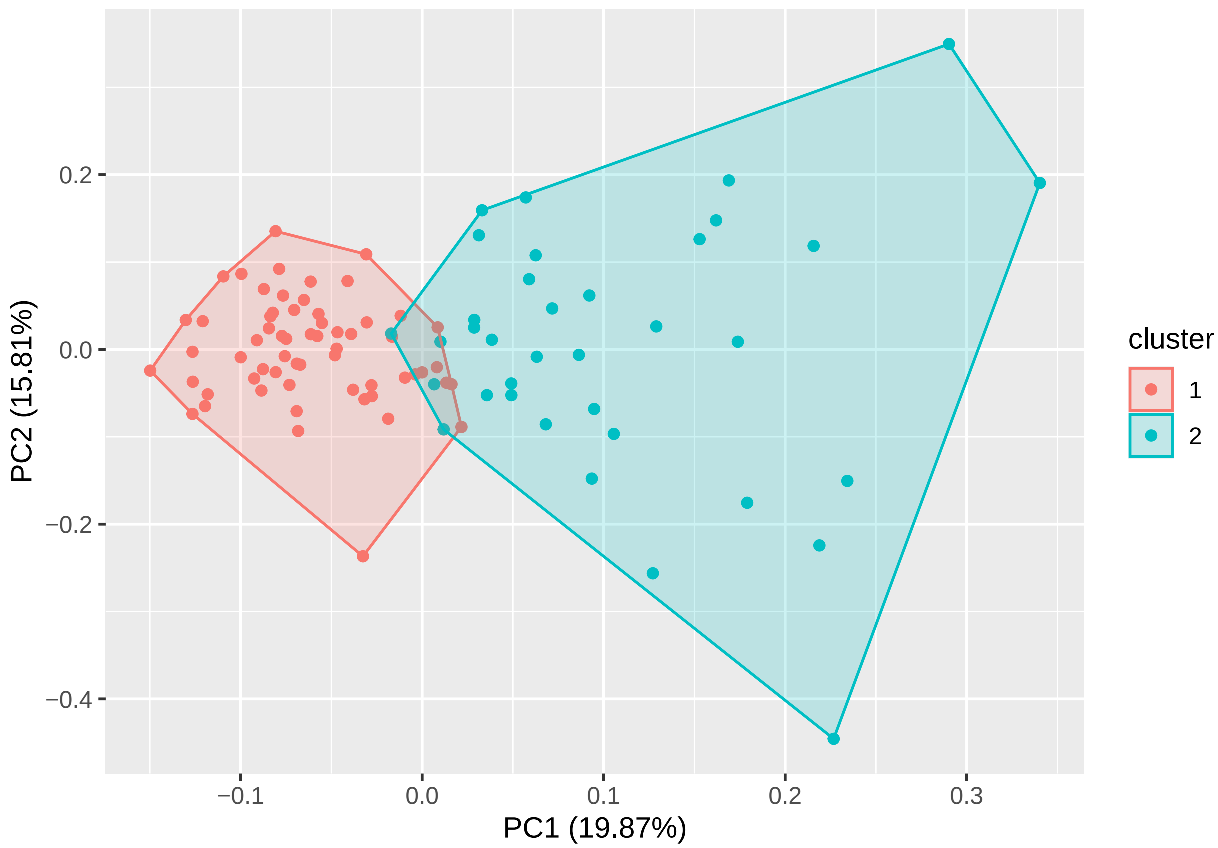

```
##  [1] 1440.0000 1239.7362 1110.7229  998.0776  941.2579  871.8389  844.3656
##  [8]  803.5112  717.8947  670.4786  617.6841  624.9956  551.0485  527.2994
## [15]  500.6391
```

```
KM_2_st <- kmeans(df_num_st[,-16], 2)
print(KM_2_st)
```

```
## K-means clustering with 2 clusters of sizes 61, 36
##
## Cluster means:
##   stearic acid   sorbitol shikimic acid    ribitol pseudo uridine nicotinamide
## 1   -0.3540244 -0.2811833    -0.2291092 -0.2903693     -0.3262451   -0.2668820
## 2    0.5998746  0.4764495     0.3882129  0.4920147      0.5528042    0.4522167
##   myo-inositol    mannose    lyxitol heptadecanoic acid glutaric acid
## 1   -0.4458068 -0.2191191 -0.3514300         -0.3588086     0.1819144
## 2    0.7553948  0.3712852  0.5954786          0.6079813    -0.3082439
##   glutamic acid citric acid behenic acid alpha-ketoglutarate
## 1    -0.2140168  -0.1714674   -0.2752593          -0.1800108
## 2     0.3626395   0.2905421    0.4664116           0.3050184
##
## Clustering vector:
##  [1] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 2 1 1 2 1 1 1 1
## [39] 1 1 1 1 2 1 1 1 1 1 2 2 1 1 1 1 1 1 2 2 2 1 1 2 2 2 2 2 1 2 2 2 1 2 2 2 2 2
## [77] 1 2 2 2 1 2 2 2 1 2 2 2 1 1 2 2 2 1 2 2 1
##
```

```
## Within cluster sum of squares by cluster:
## [1] 399.1234 837.9996
##  (between_SS / total_SS =  14.1 %)
##
## Available components:
##
## [1] "cluster"      "centers"      "totss"        "withinss"     "tot.withinss"
## [6] "betweenss"    "size"         "iter"         "ifault"
```

```
autoplot(KM_2_st, df_num_st[,-16], frame = TRUE)
```




```
KM_2_st$centers
```

```
##   stearic acid   sorbitol shikimic acid    ribitol pseudo uridine nicotinamide
## 1   -0.3540244 -0.2811833    -0.2291092 -0.2903693     -0.3262451   -0.2668820
## 2    0.5998746  0.4764495     0.3882129  0.4920147      0.5528042    0.4522167
##   myo-inositol    mannose    lyxitol heptadecanoic acid glutaric acid
## 1   -0.4458068 -0.2191191 -0.3514300         -0.3588086     0.1819144
## 2    0.7553948  0.3712852  0.5954786          0.6079813    -0.3082439
##   glutamic acid citric acid behenic acid alpha-ketoglutarate
## 1    -0.2140168  -0.1714674   -0.2752593          -0.1800108
## 2     0.3626395   0.2905421    0.4664116           0.3050184
```

```r
# SVM model with linear kernel
svm_model_linear_st <- svm(as.factor(Arm)~
                             as.numeric(tr_set_train_st$`stearic acid`) +
                             as.numeric(tr_set_train_st$sorbitol) +
                             as.numeric(tr_set_train_st$nicotinamide) +
                             as.numeric(tr_set_train_st$mannose) +
                             as.numeric(tr_set_train_st$`heptadecanoic acid`) +
                             as.numeric(tr_set_train_st$`glutaric acid`) +
                             as.numeric(tr_set_train_st$`glutamic acid`) +
                             as.numeric(tr_set_train_st$`behenic acid`),
                           data = tr_set_train_st, method = "C", kernel = "linear",
                           gamma = 1, cost = 2)
# Getting the mean of the correctly predicted arm on the train data set:
predict_tr_linear_st <- predict(svm_model_linear_st, tr_set_train_st)
mean_linear_train_st <- mean(predict_tr_linear_st == as.factor(tr_set_train_st$Arm))
mean_linear_train_st
```

```
## [1] 0.8529412
```

```r
# Getting the mean of the correctly predicted arm on the test data set:
predict_test_linear_st <- predict(svm_model_linear_st, tr_set_test_st)
mean_linear_test_st <- mean(predict_test_linear_st == tr_set_test_st$Arm)
mean_linear_test_st
```

```
## [1] 0.8529412
```

```r
# SVM model with radial kernel:
svm_model_radial_st <- svm(as.factor(Arm)~
                             as.numeric(tr_set_train_st$`stearic acid`) +
                             as.numeric(tr_set_train_st$sorbitol) +
                             as.numeric(tr_set_train_st$nicotinamide) +
                             as.numeric(tr_set_train_st$mannose) +
                             as.numeric(tr_set_train_st$`heptadecanoic acid`) +
                             as.numeric(tr_set_train_st$`glutaric acid`) +
                             as.numeric(tr_set_train_st$`glutamic acid`) +
                             as.numeric(tr_set_train_st$`behenic acid`),
                           data = tr_set_train_st, method = "C-classification",
                           kernel = "radial", gamma = 1, cost = 2)
# Getting the mean of the correctly predicted arm on the train data set:
predict_tr_radial_st <- predict(svm_model_radial_st, tr_set_train_st)
mean_radial_train_st <- mean(predict_tr_radial_st == tr_set_train_st$Arm)
mean_radial_train_st
```

```
## [1] 1
```

```r
# Getting the mean of the correctly predicted arm on the test data set:
predict_test_radial_st <- predict(svm_model_radial_st, tr_set_test_st)
mean_radial_test_st <- mean(predict_test_radial_st == tr_set_test_st$Arm)
mean_radial_test_st
```

```
## [1] 1
```

*k-NN model

In this section I will work with the standardized dataset, adn I will fit a k-NN model using this data set:

```
# Re-initializing the scaled data set used for the k-means calculation above
df_num_st <- as_tibble(scale(df_num))
df_num_st <- cbind(df_num_st, Arm = data$Arm)
index_st <- sample.split(df_num_st$Arm, SplitRatio = .7)
knn_tr <- subset(df_num_st, index == TRUE)
knn_valid <- subset(df_num_st, index == FALSE)
index_knn_tr <- sample.split(knn_tr$Arm, SplitRatio = .5)
knn_tr_train <- subset(knn_tr, index_knn_tr == TRUE)
knn_tr_test <- subset(knn_tr, index_knn_tr == FALSE)
pred_knn <- knn(knn_tr_train[,-16], knn_tr_test[,-16], knn_tr_train[,16], k = 5)
# Next we validate the predicted labels with the actual labels:
CFM_knn <- table(pred_knn, knn_tr_test[,16])
CFM_knn
```

```
##
## pred_knn             Group 1 - Score 0 Group 2 - Score 50
##   Group 1 - Score 0                 13                  6
##   Group 2 - Score 50                 4                 11
```

```
classification_accuracy_knn <- sum(diag(CFM_knn))/sum(CFM_knn)
classification_accuracy_knn
```

```
## [1] 0.7058824
```

From the above table we cn clearly see that the model did not do a very good job predicting samples from group 2. From the latter arm, 8 have been miss classified as group 1 samples and from group 1, 1 has been miss classified as group 2 sample, and 7 were correctly identified. The accuracy of the model was 68.9%.

Random Forest model

Another classification model is the Random Forest (RF) model, that is an evolved form of the Decision Trees (DTs). In this section, I will build a RF model for the 15 selected parameters. I will use the data set converted into numbers df_num. First, I will bind the 'Arm' predicted variable to the data set, then I will transform the column titles, so these do not consist of any special characters or spaces:

```
# Binding the Arm variables to the df_num data set:
df_num_RMF <- cbind(df_num, Arm = data$Arm)
# Converting the parameter names, so these do not consist space or any illegal characters:
df_colnames <- sub(' ', '_', colnames(df_num_RMF))
df_colnames <- sub('-', '_', df_colnames)
# Adding back the converted names to the column headers.
colnames(df_num_RMF) <- df_colnames
```

Next, I will create a new set of train and test sets:

```
# Creating a training and test set from the data frame df_num_RMF:
index_RMF <- sample.split(df_num_RMF$Arm, SplitRatio = .7)
tr_set_RMF <- subset(df_num_RMF, index == TRUE)
final_val_set_RMF <- subset(df_num_RMF, index == FALSE)
# Splitting the train set into further train and test sets:
index_train_RMF <- sample.split(tr_set_RMF$Arm, SplitRatio = .7)
tr_set_train_RMF <- subset(tr_set_RMF, index_train == TRUE)
tr_set_test_RMF <- subset(tr_set_RMF, index_train == FALSE)
```

Building the RMF model:

```
RMF <- randomForest(as.factor(Arm)~sorbitol +
                      nicotinamide +
                      stearic_acid +
                      mannose +
                      heptadecanoic_acid +
                      glutaric_acid +
                      glutamic_acid +
                      behenic_acid,
                    data = tr_set_train_RMF)

# Now that we have built the RMF model, we use the predict function to get the
# predicted values for the data set:
predict_RMF_tr <- predict(RMF, tr_set_train_RMF)
# Adding the predicted arms to the data set:
tr_set_train_RMF$predict_RMF_tr <- predict_RMF_tr
# Compare the predictions and the actual values to see the accuracy of the model
# using the table() function (building a confusion matrix):
CFM_tr <- table(tr_set_train_RMF$Arm, tr_set_train_RMF$predict_RMF_tr)
CFM_tr
```

```
##
##                       Group 1 - Score 0 Group 2 - Score 50
##   Group 1 - Score 0                  17                  0
##   Group 2 - Score 50                  0                 17
```

```
# Calculating the accuracy of the testing data can be measured by adding together
# all the diagonal values, and dividing with the sum of all values:
classification_accuracy_tr <- sum(diag(CFM_tr)/sum(CFM_tr))
classification_accuracy_tr
```

```
## [1] 1
```

Testing the model on the test set:

```
predict_RMF_test <- predict(RMF, tr_set_test_RMF)
tr_set_test_RMF$predict_RMF_test <- predict_RMF_test
CFM_test <- table(tr_set_test_RMF$Arm, tr_set_test_RMF$predict_RMF_test)
CFM_test
```

```
##
```

```
##                        Group 1 - Score 0 Group 2 - Score 50
##   Group 1 - Score 0                  11                   6
##   Group 2 - Score 50                  6                  11
```

```
classification_accuracy_test <- sum(diag(CFM_test)/sum(CFM_test))
classification_accuracy_test
```

```
## [1] 0.6470588
```

A standardized data has also been tested for building an RMF model, however, this also led to a low accuracy.

Validation

SVM

Validating the SVM model (linear and radial)

```
# Non-standardized Values - Linear
predict_valid_linear <- predict(svm_model_linear, final_val_set)
mean_linear_valid <- mean(predict_valid_linear == final_val_set$Arm)
mean_linear_valid
```

```
## [1] 0.7647059
```

```
# Non-standardized Values - Radial
predict_valid_radial <- predict(svm_model_radial, final_val_set)
mean_radial_valid <- mean(predict_valid_radial == final_val_set$Arm)
mean_radial_valid
```

```
## [1] 0.7647059
```

```
# Standardized Values - Linear
predict_valid_linear_st <- predict(svm_model_linear_st, final_val_set_st)
mean_linear_valid_st <- mean(predict_valid_linear_st == final_val_set_st$Arm)
mean_linear_valid_st
```

```
## [1] 0.6764706
```

```
# Standardized Values - Radial
predict_valid_radial_st <- predict(svm_model_radial_st, final_val_set_st)
mean_radial_valid_st <- mean(predict_valid_radial_st == final_val_set_st$Arm)
mean_radial_valid_st
```

```
## [1] 0.7647059
```

KNN

```r
pred_knn_valid <- knn(knn_tr_train[,-16], knn_valid[,-16], knn_tr_train[,16], k = 5)
# Next we validate the predicted labels with the actual labels:
CFM_knn_valid <- table(pred_knn_valid, knn_valid[,16])
CFM_knn_valid
```

```
##
## pred_knn_valid       Group 1 - Score 0 Group 2 - Score 50
##   Group 1 - Score 0                 12                  7
##   Group 2 - Score 50                 2                  8
```

```r
classification_accuracy_knn_valid <- sum(diag(CFM_knn_valid))/sum(CFM_knn_valid)
classification_accuracy_knn_valid
```

```
## [1] 0.6896552
```

RMF

Validating the RMF model:

```r
predict_valid_RMF <- predict(RMF, final_val_set_RMF)
mean_RMF_valid <- mean(predict_valid_RMF == final_val_set_RMF$Arm)
mean_RMF_valid
```

```
## [1] 0.6551724
```

```r
final_val_set_RMF$predict_valid_RMF <- predict_valid_RMF
CFM_val <- table(final_val_set_RMF$Arm, final_val_set_RMF$predict_valid_RMF)
CFM_val
```

```
##
##                      Group 1 - Score 0 Group 2 - Score 50
##   Group 1 - Score 0                 11                  3
##   Group 2 - Score 50                 7                  8
```

```r
classification_accuracy_val <- sum(diag(CFM_val)/sum(CFM_val))
classification_accuracy_val
```

```
## [1] 0.6551724
```

Results and conclusions

```r
model_results_tibble <- tibble(Models = c("SVM Linear","SVM Radial",
                                          "SVM Standardized Linear",
                                          "SVM Standardized Radial",
                                          "RMF", "KNN"),
                               TrainFit = c(mean_linear_train, mean_radial_train,
```

```r
                                            mean_linear_train_st, mean_radial_train_st,
                                            classification_accuracy_tr,
                                            '-'),
                              TestFit = c(mean_linear_test, mean_radial_test,
                                          mean_linear_test_st, mean_radial_test_st,
                                          classification_accuracy_test,
                                          classification_accuracy_knn),
                              Validation = c(mean_linear_valid, mean_radial_valid,
                                             mean_linear_valid_st, mean_radial_valid_st,
                                             classification_accuracy_val,
                                             classification_accuracy_knn_valid)) %>%
  mutate(TestFit = sprintf("%0.4f", TestFit))

model_results_tibble
```

```
## # A tibble: 6 x 4
##   Models                  TrainFit          TestFit Validation
##   <chr>                   <chr>             <chr>        <dbl>
## 1 SVM Linear              0.823529411764706 0.8235       0.765
## 2 SVM Radial              1                 1.0000       0.765
## 3 SVM Standardized Linear 0.852941176470588 0.8529       0.676
## 4 SVM Standardized Radial 1                 1.0000       0.765
## 5 RMF                     1                 0.6471       0.655
## 6 KNN                     -                 0.7059       0.690
```

Future perspectives