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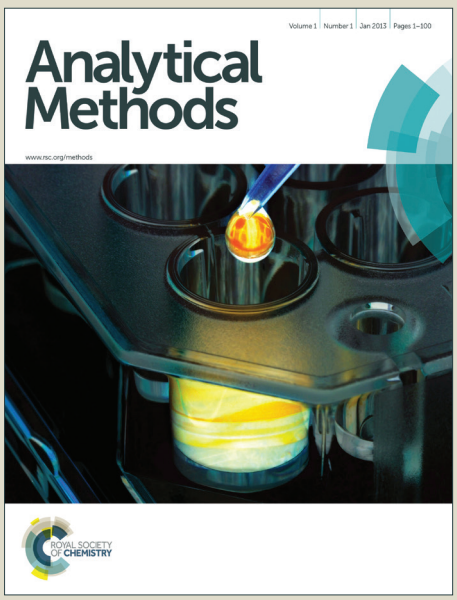

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Rapid adulteration detection for flaxseed oil using ion mobility spectrometry and chemometric methods

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Abstract

To prevent potential adulteration of flaxseed oil with high amounts of nutritional components, a simple and rapid adulteration detection method was proposed based on ion mobility spectrometry (IMS). After dilution in n-hexane, the edible oil sample was analyzed by IMS for 20 s. Subsequently, the multivariate statistical methods including principal component analysis (PCA) and recursive support vector machine (R-SVM) were employed to establish a discriminant model for authentic and adulterated flaxseed oils. The cross validation results indicated that the R-SVM model could identify adulterated flaxseed oil samples (≥ 5%) with high accuracy of 93.1%. Therefore, IMS could be used as an important tool to protect customers from adulterated flaxseed oil.

Keywords

Flaxseed oil; rapid adulteration detection; ion mobility spectrometry; chemometrics

1. Introduction

Flaxseed oil, sometimes called linseed oil, is made from the seeds of the flax plant (*Linum usitatissimum*, *Linaceae*). With high amounts of nutritional components such as essential omega-3 fatty acids and phytoestrogen lignans, flaxseed oil has become popular edible oil in the health food market in countries including China, India, and Canada. Omega-3 fatty acids in flaxseed oil have been reported to reduce risk factors associated with inflammatory diseases, cardiovascular diseases, and cancers.[1-3] Besides, lignans in flaxseed and flaxseed oil show potential anti-estrogenic effects on estrogen receptor-positive breast cancer, colon cancer, and prostate cancer. Therefore, dietary flaxseed or flaxseed oil has the potential to reduce tumor growth in patients with these cancers.[4-6] In addition, flaxseed oil contains all essential amino acids that are crucial for synthesis of the proteins that regulate and maintain proper cellular functions.[7] As a result, flaxseed oil has gained popularity over the last two decades in the diets of people because of perceived improvements to human nutrition and health status.[8, 9] Meanwhile, flaxseed or flaxseed oil has also been employed as an important additive in functional foods[9] and as feeds for livestock, which enhances the nutritional quality of related products.[10-12]

Food ingredient fraud and economically motivated adulteration are emerging risks that challenge human health. The associated database demonstrates that oil fraud is the most common target for food adulteration, accounting for about 24% of all food fraud records in the scholarly database.[13] Authenticity assessment of edible vegetable oils is a tough nut to crack worldwide. Similarly, with olive oil adulteration in western countries, adulteration of high-price oils like flaxseed oil is also a kind of agricultural fraud found all over the world. Therefore, oil adulteration detection is highly demanded.[14] The most common instrumental detection methods include liquid

chromatography[15] or gas chromatography[16] coupled with mass spectrometry[17], infrared spectroscopy,[18] fluorescence spectroscopy,[19] or Raman spectroscopy.[20]

Ion mobility spectrometry (IMS) is an analytical technique for determination of volatile and semi-volatile compounds based on gas-phase separation of the resulting ions in a weak electric field under ambient pressure.[21] Because of pretreatment-free detection of samples, operating convenience and short analysis time, IMS was first known as the best method for screening explosives at airport, detecting chemical warfare agents for military and monitoring stack gas emissions.[22] Recently, the use of IMS is increasing in wider areas such as food and agro-food safety[23, 24] and quality assurance and process monitoring in the pharmaceutical industry.[25] Moreover, volatile organic compounds (VOCs) in breath were rapidly analyzed by IMS to detect sepsis in rats,[26] and bacterial fingerprinting in IMS analysis was used for classification and differentiation of specific strains and species of bacteria.[27] IMS was also used as a rapid and sensitive on-site method to detect microbial volatile organic compounds (MVOCs) for indicating actively growing fungi concealed within wood.[28] Recently, IMS coupled with chromatographic column was employed to classify three types of olive oils[29-31].

Chemometrics is a multivariate data analysis tool often used to select the most important variables and establish a good predictive model. In respect to detection of oil fraud, chemometrics is used to qualitatively identify the adulterated edible oils and quantitatively determining adulterants in oil samples.[31] Some recent reports showed the use of chemometric methods such as principal component analysis (PCA), hierarchical cluster analysis (HCA), self-organizing maps based on chaotic parameters, and cluster discriminant analysis (CDA) in distinguishing edible oils from refined recycled cooking oils, identifying edible oils from different regions, and detecting

adulteration of extra virgin olive oil with inferior edible oils.[17, 32, 33]

In this study, edible vegetable oils were analyzed by IMS. Subsequently, a classification model for flaxseed oil and another five types of edible oil, and a discriminant model for flaxseed oil and its simulated adulterated oils were constructed by PCA and recursive support vector machine (R-SVM) for detecting adulterated flaxseed oil.

2. Materials and methods

2.1. Oil samples and reagents

Among four vegetable oils used in this study, 20 virgin flaxseed and 17 soybean oils were from the seeds, while 22 refined flaxseed, 8 cottonseed and palm oils were acquired at stores. 10 flaxseed and 17 soybean samples were purchased from different regions. Each flaxseed sample was divided into two, and one of them was dried at 60℃ for 4 h in a thermostat oven. Then, 20 flaxseed and 17 soybean samples were squeezed using a TEN GUARD oil pressing machine (TZC-0502, made in China). Meanwhile, 22 flaxseed oil, 8 cottonseed oil and 2 palm oil samples were purchased from the local market. Also, 2,4,6-trimethylpyridine (≥ 99.0% purity) was purchased from Sigma (St. Louis, MO, USA). n-Hexane (HPLC grade) was obtained from Anhui Fulltime Specialized Solvent & Reagent Co., Ltd (China).

2.2. Sample preparation

Virgin flaxseed oil was randomly selected and adulterated by 5%, 10%, 20%, 30%, 40%, and 50% (v/v) with palm oil, cottonseed oil, and soybean oil in duplicate, respectively. Totally, 36 adulterated oil samples were prepared. Then, 100 μL oil was diluted by n-hexane to 5 mL and well mixed by vortex for 30 s for subsequent IMS analysis.

2.3. IMS analysis

IMS analysis was performed in this study by an ion mobility spectrometer (IMS-KS-100) from Wuhan Syscan Technology Co., Ltd, which was based on a pulsed glow discharge ionization source working in negative mode. Carrier and drift gases were both dried air, and Table 1 lists detailed IMS parameters. Using this device, reduced mobility K0 at 1.81 ± 0.005 $cm^2/(s \cdot V)$ was determined for the reference compound (2, 4, 6-trimethylpyridine) that could be used for instrument calibration.

(Insert Table 1)

2.4. Multivariate analysis

The IMS data of edible oils were acquired through 60 scans in analysis time of 20 s. Therefore, the IMS data of one edible oil is two-dimensional matrix (detection time and drift time). The sub-matrix was extracted to eliminate signal interference from the solvent of n-hexane. The IMS spectrum of each edible oil sample was created by summing the intensities at the same drift time and normalized by dividing the maximum value.

The data matrix included the IMS spectra of 22 flaxseed oils and 36 adulterated oils. The data matrix was preprocessed by generalized log2 transformation and Pareto scaling (mean-centered and divided by the square root of the standard deviation of each variable). As exploratory data analysis, PCA was employed to examine the sampling clusters and variable distributions. Then, a discriminant model was established for flaxseed oil and its adulterated oils by R-SVM,[34] and the discriminant model was validated by 10-fold cross validation.

The data were processed on a Pentium 4 personal computer running Windows 7. The programs of the automatic search tool for straight saturated fatty acid methyl esters (FAMEs) and the calculation of theoretical and experimental equivalent chain length (ECL) values were coded

in Matlab 2011a for Windows (The Mathworks, Natick, MA). Data preprocessing (Pareto scaling), clustering (PCA and HCA), and classification (R-SVM) were conducted by a metabolomic data analysis tool MetaboAnalyst 2.0.[35,36]

3. Results and discussion

3.1 Rapid analysis of flaxseed oil by ion mobility spectrometry

Flaxseed oil has won popularity in the health food market because of its high amounts of nutritional components such as essential omega-3 fatty acids and phytoestrogen lignans. Aroma is an important quality criterion for edible vegetable oils as a characteristic parameter.[37] Our recent studies indicated that VOCs were important markers for edible oils.[38, 39] Meanwhile, it was found that four edible oils including soybean, peanut, rapeseed and sunflower seed oils had their own characteristic VOCs. In this case, a method for rapid adulteration detection of edible oils could be established and standardized to prevent oil adulteration if these VOCs could be swiftly detected. For rapid VOC analysis, electronic noses, IMS and their combination with gas chromatography are the best choices. In this study, IMS was employed to develop a rapid adulteration detection method for flaxseed oil.

Among the IMS parameters, the inlet temperature is the most important one. Generally, higher inlet temperature indicates more VOCs emerging from oils and input into ion sources, as well as more information in IMS spectra. However, if the inlet temperature is higher than the smoke point of this edible oil, a bluish smoke becomes clearly visible. Therefore, the inlet temperature was set to 170ºC, lower than the smoke points of most edible oils. Meanwhile, since the IMS spectrum is nearly invariable after 20 s, the analysis time was set to 20 s. Therefore, each edible oil sample was diluted by n-hexane and analyzed by IMS for 20 s at the optimized

conditions.

(Insert Figure 1)

Apart from analysis of several known markers elsewhere, IMS was employed to analyze the whole response of edible oils. Due to the contraceptive property of gossypol and low cost genetically modified materials, cottonseed and soybean oils have relatively low prices in China, leading them to become adulterants in high price edible oils. Meanwhile, high contents of saturated fatty acids make palm oil less popular as cooking oil. As a consequence, these three vegetable oils were selected as potential adulterants for flaxseed oil. In Figure 1, the typical IMS spectra of pure flaxseed, cottonseed and soybean oils were illustrated. Obviously, differences exist among the three spectra especially at the drift time of 12.0-15.5 ms. This result indicates that cottonseed oil possesses more small molecular VOCs at the inlet temperature of 170ºC. Though the IMS spectra of the same kind of edible oil vary, the spectra could reflect the differences among these three vegetable oils (see Figure 2). In the PCA score plot (PC 2 vs. PC 4) in Figure 2, we also found that the differences between extra virgin and refined flaxseed oils were smaller than the differences among these three vegetable oils. These results indicate that IMS spectra could represent edible oils and be employed for quality control.

3.2 Exploratory data analysis

From the above analysis, we could find that the IMS spectra of the three vegetable oils could be classified into three groups. In the following sections, adulteration detection was investigated for flaxseed oil based on the IMS spectra. As described in section 2.2, 36 adulterated oils were prepared and analyzed by IMS. The data matrix of the IMS spectra of pure and adulterated flaxseed oils was preprocessed using generalized log transformation and Pareto scaling. At first,

PCA was used to show the clustering of samples in pure and adulterated flaxseed oils. The score plot in Figure 3 showed that the IMS spectra of pure and adulterated flaxseed oils were overlapped and also showed a separation tendency, as displayed on the 1st and 3rd PCs. Therefore, it is necessary to conduct variable selection and establish a discriminant model by supervised data analysis methods.

(Figure 3)

3.3 Discriminant model for adulteration detection

In this study, to predict whether labeled flaxseed oils were adulterated with soybean, cottonseed and/or palm oils, a discriminant model was established after generalized log transformation and Pareto scaling. Taking the sample balance in supervised leaning into account, 36 adulterated oils were prepared by adulterating palm oil, cottonseed oil, and soybean oil in duplicate into pure flaxseed oils by 5%, 10%, 20%, 30%, 40%, and 50% (v/v), respectively. Then, the discriminative model was built for pure and adulterated flaxseed oils by R-SVM.

Recently, R-SVM was proposed to select important genes or biomarkers to classify noisy data in Bioinformatics.[34] R-SVM recursively classify the samples with SVM with linear kernel and select important variables according to discriminatory power between the two classes. Classification model was recursively built using different feature subsets. Features are selected based on their relative contribution in the classification using cross validation error rates. The detailed algorithm of R-SVM was described elsewhere [34]. Herein, R-SVM recursively classified pure and adulterated flaxseed oils using SVM with a linear kernel and selected important variables based on the discriminatory power. The classification model was recursively built using different feature subsets. The least important variables were eliminated in subsequent steps. Then,

important variables were selected according to their relative contributions to the classification using cross-validation error rates. In this process, a series of SVM models were created and their cross validation error rates were shown in Figure 4. Finally, the model that used the minimal number of features and also gave the minimal 10-fold cross-validation error rate was selected as the final model.

(Figure 4)

As shown in Figure 4a, the best classifier was built with the whole spectra (650 variables). The 10-fold cross-validation error rates revealed that the error rate of 6.9% was obtained to discriminate pure flaxseed oils from adulterated ones when the 650 variables were used in the SVM model. Along with the decrease in the number of variables, the error rate decreased. As shown in Figure 4b, the 15 most important variables were the responses at the drift time from 11.726 ms to 12.090 ms, which could provide a prediction correction rate of about 76%. This result is consistent with the result in Figure 1, indicating that small molecular VOCs might be markers for adulteration with soybean, cottonseed and palm oils. Since there are various VOCs in edible oils, the IMS spectra cannot be clearly interpreted by the chemical composition. Thus, further combination with gas chromatography and sample preparation techniques such as solid-phase microextraction (SPME) might help the interpretation of IMS spectra of edible oils and provide a more effective model for adulteration detection.

4. Conclusion

Flaxseed oil has become increasingly popular due to its high amounts of nutritional components including essential fatty acids and phytoestrogen lignans. Therefore, it possesses a high risk of being adulterated with other low-price edible oils. To date, there is still no method for

rapidly detecting flaxseed oil adulteration. In this study, a rapid method of adulteration detection for flaxseed oil was proposed using ion mobility spectrometry (IMS). The IMS analysis of edible oil samples could be conducted after dilution in n-hexane. The analysis time is just 20 s. A discriminant model for identifying the adulterated flaxseed oils was established by R-SVM. The cross validation results indicated that the discriminant model built with the IMS spectra could identify adulterated flaxseed oil samples (≥ 5%) with high accuracy of 93.1%. As a result, IMS might be an important tool to protect customers from adulterated flaxseed oil.

Acknowledgments

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Conflict of interest

The authors have declared that no competing interests exist.

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Figure captions:

Figure 1 IMS spectra of flaxseed, cottonseed, soybean oils

Figure 2 PCA score plot for flaxseed, cottonseed, soybean oils (PC2 vs PC4)

Figure 3 PCA score plot for pure and adulterated flaxseed oils

Figure 4 (a) Recursive classification with SVM. The red circle indicates the best classifier; (b) Significant features identified by R-SVM and ranked by their frequencies of being selected in the classifier.

Table title:

Table 1 The IMS parameters used in this study

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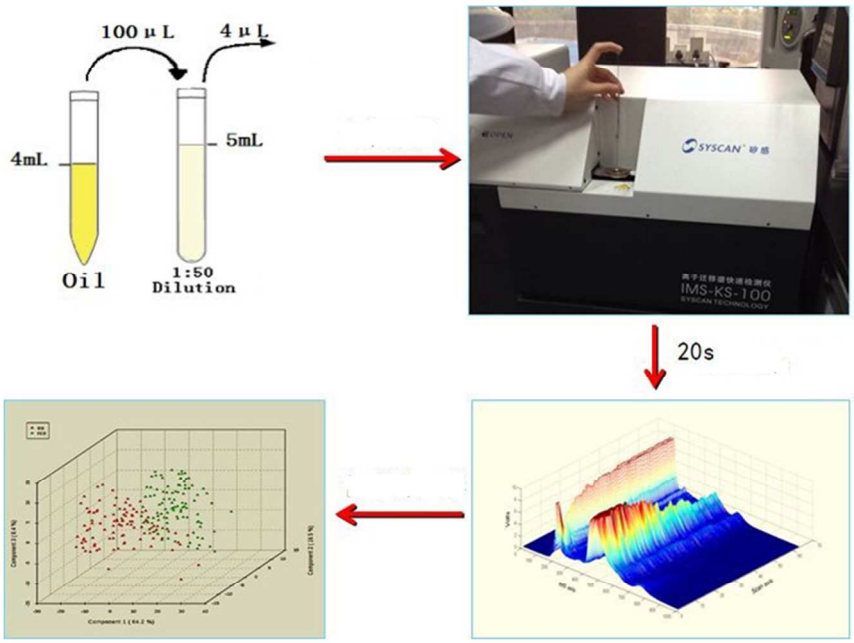
Parameters	Setting
Ion Source	PGB[1]
Drift field (V /cm)	300
Drift gas flow (mL/ min)	700
Carrier gas flow (mL/ min)	300
Drift tube temperature (℃)	60
Inlet temperature (℃)	170
Drift tube length (cm)	15
Analysis time (s)	20
Discharge time(μs)	676
Sampling frequency(scans/s)	16
Gate pulse width (μs)	100

[1] PGB：Pulse glow discharge



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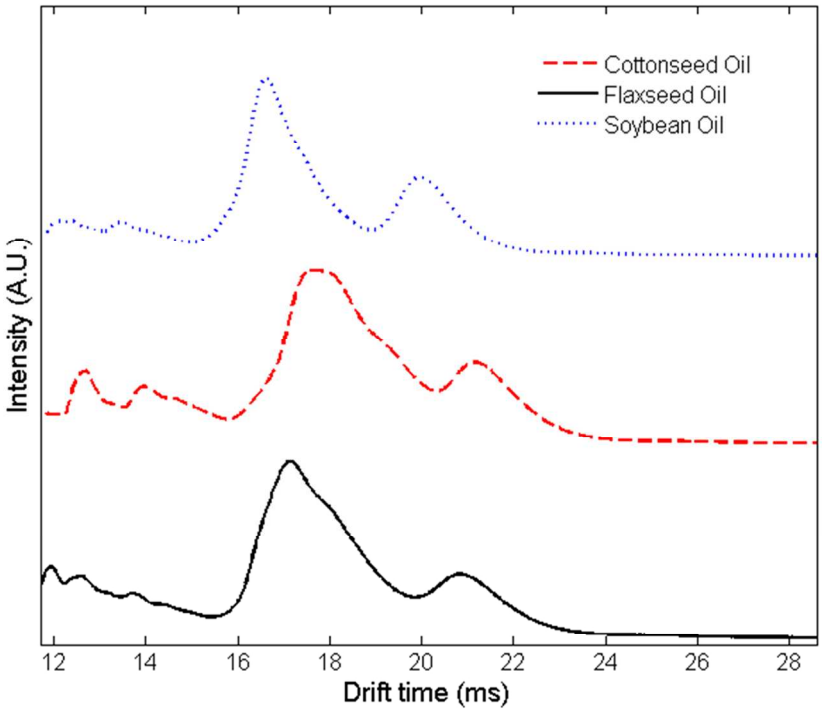

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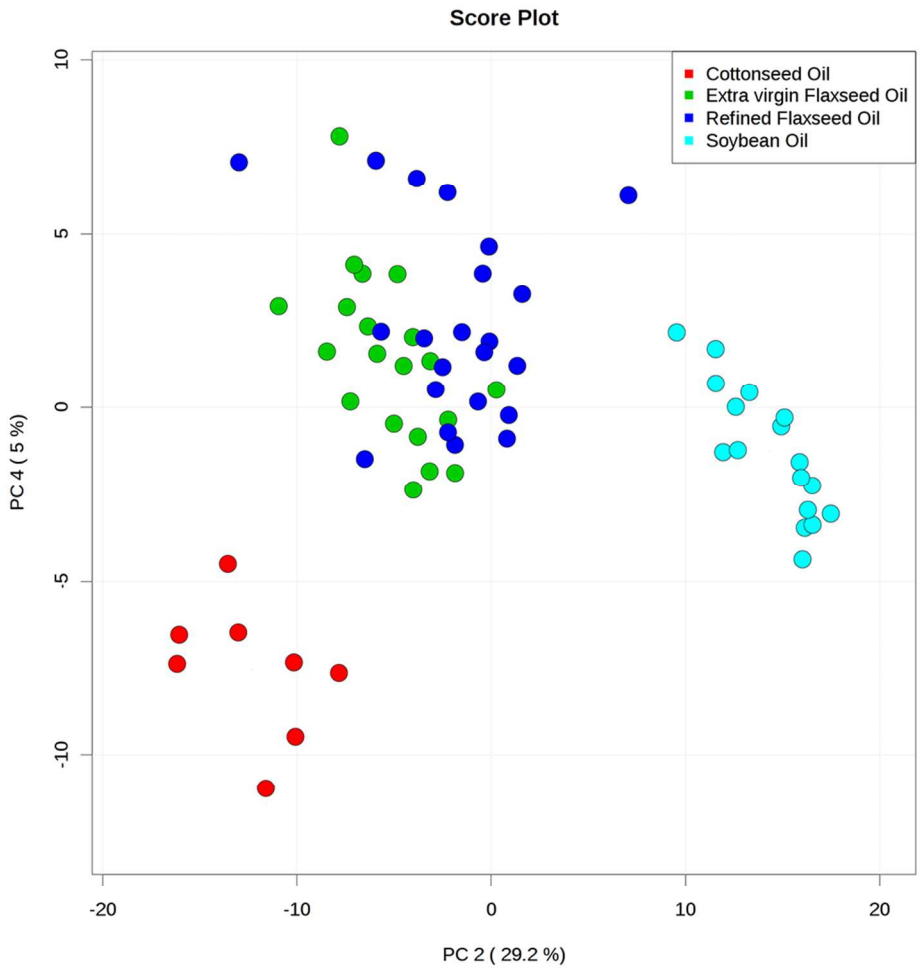

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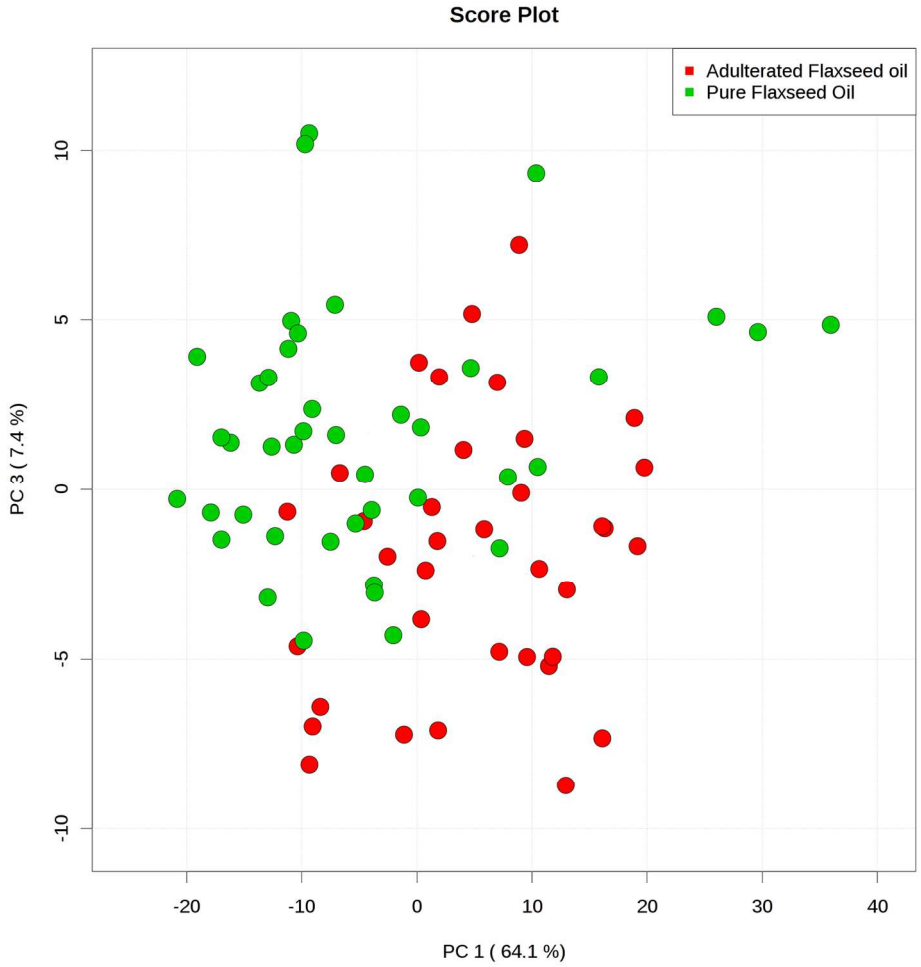

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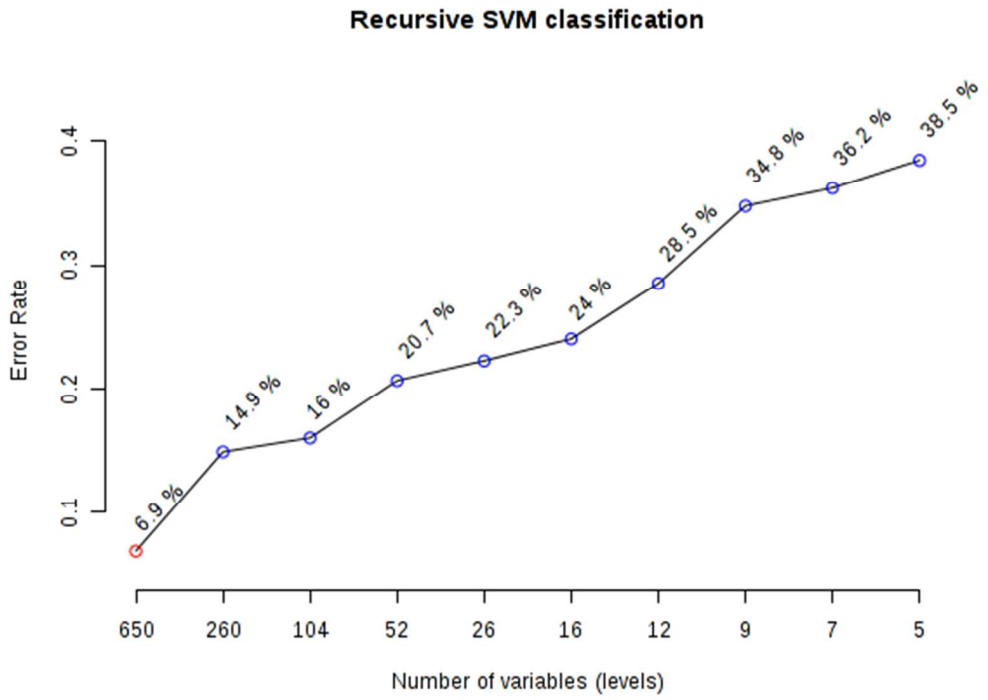

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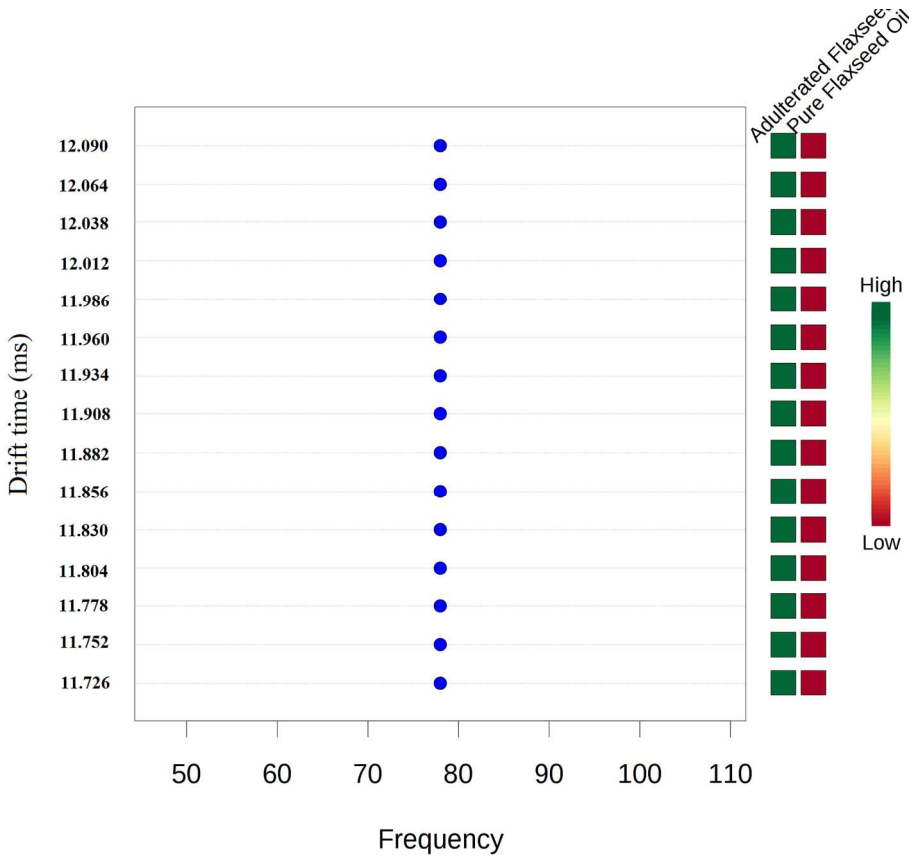

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