**{, #38}Predicting fungal infection sensitivity of sepals in harvested tomatoes using Imaging Spectroscopy and Partial Least Squares Discriminant Analysis**

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1. **Introduction**

Tomato (Solanum lycopersicum L.) is an "ubiquitous vegetable". Tomatoes are produced globally, either for domestic consumption or as a commodity for international export. The nutritional composition of this fruit includes carbohydrates, lipids and proteins. In addition, it contains vitamins, minerals, and carotenes in smaller proportions. {, #1}

Tomato quality is divided into different aspects, commercial, organoleptic and nutritional {Nadia Bertin, 2018 #2}. Market standard primarily depends on the external appeal (e.g. color, form, size), firmness and shelf life, {, #38}Regulation (EU) No 543/2011, CODEX STAN 293-2008 , UNECE [Standard FFV-36](https://unece.org/trade/wp7/FFV-Standards)  whereas health benefits rely on relies on the nutritional value as well as on the absence of pathogenic hazards or contaminants {Nadia Bertin, 2018 #2}. Pathogenic fungi can infect and spread to many different parts of a tomato plant, including the stem, calyx and skin of the fruit {E.J. Smid, 1996 #19}. The portion of tomatoes that go to waste after the harvesting stage can reach 42% worldwide (Kojo at al. 2015). Around 30% of the harvested tomato produce may be lost during postharvest handling, primarily because of microbial decay caused by fungi such as *Rhizopus stolonifer, Alternaria alternata, and Botrytis cinerea* (Peralta-Ruiz et. Al, 2020). In some countries tomatoes are sold including calyx. Fresh looking green parts of a tomato (calyx and vine) are a sign for dealing with fresh tomatoes. Older tomatoes show dehydration symptoms of the green parts. The calyx is also susceptible to infection by fungal spores. These spores may already be present on the tomato during cultivation. After harvest, under humid and poorly ventilated storage and transport conditions, these spores may germinate and grow further into visible mould on the calyx{Manon Mensink, 2019 #21}. This negatively affects the value of the fruit and may lead to extra food loss and waste{Jan Janse, 2007 #20}{Manon Mensink, 2019 #21}.

The timely identification of disease has the potential to avert losses since prompt actions can be implemented to mitigate more extensive damages (e.g. adapt packing strategies{Manon Mensink, 2019 #21}. Generally, the strategy employed in the industry to reduce pathogen attacks is the use of pesticides. However, these products can damage the food and diminish its nutritional value {Nadia Bertin, 2018 #2}. Whenever possible, it is preferable to protect the harvested fruits by using methods that do not introduce any additional chemicals or contaminants and do not harm the food in any way.

A possible means to assess the predisposition to microscopic fungal contamination is by tracking the growing and handling conditions of tomato produce within the supply chain. This correlation may be beneficial in the detection of probable origins of fungal contamination based on historical data. However, tracking of individual tomatoes or even batches from growth to harvest and later post-harvest handling and logistics is highly difficult.

Some tomatoes are more susceptible for infection and growth of spores while others are not (cite Brdar et al). Moreover, susceptibility of individual sepals also differs. It is not known yet, what is causing this difference. This knowledge would be useful to predict the susceptibility to this infection and growth. A more specific method is necessary which allows each calyx and sepal to be evaluated individually.

Some of the analytical methods traditionally used to evaluate the presence of fungus in plants are summarized here. Firstly, new DNA-based technology has been developed to support and replace morphology-based detections of phytopathogenic fungi. Daniel Jiménez, in 2009, developed a real-time qPCR assay for the calculation of F. oxysporum DNA in plant tissues and soil {Daniel Jiménez-Fernández, 2009 #4}. Moreover, tomato samples can be tested for mycotoxins, as a high level of these compounds is caused by fungal infection {Mina Nan, 2022 #5}. Some detection solutions are, for instance, chromatography coupled with detector methods (Delavy et al., 2019), electrochemical biosensors technology ([Dyussembayev et al. 2021)](https://www.frontiersin.org/people/u/1147699) and immunological techniques such as such enzyme-linked immunosorbent assay (ELISA, Rajeshwari et al. 1998) ),dipsticks and flow-through membranes (Shuodan Hu et al. 2023). Furthermore, chromatography-mass spectrometry (GC-MS) or electronic nose (e-nose) can be used to measure the shift of the composition and concentration of volatile organic compounds (VOCs) emitted by diseased tomatoes {Mina Nan, 2022 #5}.

Although these analytical methods are specific and accurate, they have several disadvantages. First of all, most of them destroy the sample during measurements. Furthermore, they are methods for detecting disease symptoms and not the susceptibility to fungal infection and growth. That is to say, they evaluate what is happening to the fruit exactly at the moment of the analysis. In the case of visible symptoms of the fungus, the future is already known (this state will continue and worsen in the future); however, if the fruits are not yet infected or the fungi has not germinated, these methods cannot predict what will happen to them in the future.

There is a need for a reliable, non-destructive and specific method to predict susceptibility to fungal infection in a rapid manner. This would provide additional support for quality inspectors.

Infrared spectroscopy can provide a possible solution to this problem. Paul Skolik et al, in 2019, have studied diseased progression in whole tomatoes using AFT-FTIR and have highlighted that plant-pathogen interaction can be identified through alteration in the spectra fingerprint {Paul Skolik, 2019 #6}

Imaging Spectroscopy (or “hyperspectral imaging”, “HSI”) can be even more useful, because spectral information can be captured across the complete product at pixel level. Huting Wang et al. 2021 used spectral imaging to detect early decay in tomatoes {Huting Wang, 2021 #7}

With similar motivation as the current article, in a previous study, Brdar et al. Investigated ensemble based machine learning methods, for early detection of sepals’ sensitivity to fungal infections on one tomato cultivar (Brioso) {Sanja Brdar, 2022 #8}. On the one hand, their research results need to be extended to multiple cultivars. On the other hand, there is a need to investigate traditional chemometric approaches to, for instance feature selection, before training the models. To the best of our knowledge, no prior research applies HSI together with chemometric analysis to predict susceptibility to fungal infection of recent harvested tomatoes.

The objective of our research was to investigate whether it was possible to predict the susceptibility of freshly harvested tomatoes to fungal infections using HSI together with chemometrics.

The methodology used for this aim included spectra extraction from HSI images, and model calibration and validation using Partial Least Squares Discriminant Analysis (PLSDA), focusing on the optimization of the model parameters.

**2) Background**

**2.1. Feature selection**

In constructing chemometric models, it is recommended to carefully select variables ({Michael D. Sorochan Armstrong, 2022 #10}*)* (which refers to wavelengths or wavenumbers in spectroscopy). It is preferable to identify and incorporate variables that significantly contribute to the studied phenomenon rather than include hundreds or thousands of variables blindly. While certain methods, like Partial Least Squares (PLS), can tolerate variable redundancy, understanding the importance of individual variables improves overall model effectiveness as well as model interpretability.

Furthermore, if a model is built for routine use, it is generally optimal to measure only some variables in the unknown, new samples. When it comes to NIR spectroscopy, there are two main challenges: 1) It is important to limit the outcome space, since this is directly proportional to the number of variables in a dataset {J.M. Roger, 2011 #12} and 2) If variables are strongly correlated, the model carries a risk of overfitting. The use of a simple model, which has fewer, but complementary, variables has a better chance of being generalized to a new sample than a model that has hundreds of highly correlated variables.

It is also important to note that some variables are important in combination with others. A variable could have average individual interest but, together with other variables, can gather important information related to a given problem.

The feature selection methods can be classified as Filters, Embedded and Wrapper, according to the interconnection with the problem to be solved ({Michael D. Sorochan Armstrong, 2022 #10} ).

Filter methods are used optionally, before wrapper and embedded methods. The user provides a threshold of feature importance, above which variables are retained in the model. These methods are univariate, and do not directly depend on the performance of the final models. ({Michael D. Sorochan Armstrong, 2022 #10} ).

When Embedded methods are used, variable selection is carried out at the same time as learning {Nicholas Pudjihartono, 2022 #9} by cross-validation performance.

On the other hand, wrapper methods iteratively choose a subset of features, train the models, and choose then the best combination. They use the model's error rate on a holding set to score feature subsets {Michael D. Sorochan Armstrong, 2022 #10}

In previous works, embedded methods (Random Forest) were used to solve a similar research problem {Sanja Brdar, 2022 #8}. This is why, in the present study, a wrapper method (the CovSel algorithm {J.M. Roger, 2011 #12}) was chosen to compare results and to evaluate the performance of a different approach.

When using the CovSel algorithm, the significance of each wavelength is assessed by calculating the covariance between each variable and the Y values (responses) {Alessandra Biancolillo, 2019 #11}. Firstly, X and Y matrices are centered (the mean value is deleted from each individual value in each dataset, as a result, the mean value in each dataset becomes zero). Then, the covariance of all the variables in X is calculated, with respect to the response Y, and the variable with the highest covariance is chosen. Then, all remaining variables are orthogonalized with respect to the chosen variable, and the procedure is repeated until the number of variables chosen by the user is completed{J.M. Roger, 2011 #12})

2.2. **Outlier removal**

There are two typical ways to proceed when analyzing spectra extracted from spectral images. One is to merge all the spectra (pixels) corresponding to a region of interest (ROI), in our case a sepal. The other is to directly make the predictions at the level of each pixel. The first option allows to drastically reduce the dimensionality of the data by reducing the number of spectra (rows), which consequently reduces the analysis time. In this work, this first option was chosen, that is to say, all the pixels coming from the ROI were merged leading to a single spectrum per ROI.

It is important to mention that not all pixels found in a ROI provide relevant information. Some pixels can be “noisy” or “bad/abnormal pixels”. According to Chu Zhang et al, in 2020, random errors are primarily generated by the surrounding conditions and the devices themselves *{Chu Zhang a b, 2020 #13}.* In 2009, James Burger found that bad pixels exhibit significantly different spectra compared to their neighbors Burger, 2009 #22}. He classified these abnormal pixels into "dead pixels," which do not respond to light, "hot pixels," characterized by high dark current, and "stuck pixels," which maintain an almost constant intermediate value. In 2023, Bingkai Liu further explained that hot pixels have a higher dark current than normal pixels, which experience a moderate dark current increase after irradiation {Bingkai Liu, 2023 #23}.

Moreover, some pixels are always noisy, while others only sporadically; some may show a “*non-linear response to light intensity*”{Burger, 2009 #16}, while some others behave randomly.

In any case, these abnormal pixels behave differently from the rest and should be eliminated. In this work, they were detected in an unsupervised manner (see Section x.y.z for details).

2.3. Classification methods

There are two types of classification methods, those called "class modeling" and those called "simple discrimination".

The first type of methods create a model for each studied class. The focus of the methods remains on one individual class at a time. The samples in each class fall into a closed class space. Moreover, the shape of the space is characteristic of the class modeling method used whether the width of the class space is determined by the confidence level applied.

An object can be assigned to one class, to more than one class, or not assigned (outlier). Examples of these are Soft Independent Modeling of Class Analogies (SIMCA) (Ballabio 2013); Potential function methods (POTFUN) (Forina, 1991) and Unequal variance methods (UNEQ) Massart 1986).

On the other hand, simple discrimination methods create a boundary between classes, so that they always assign each object to the class to which it most likely belongs. The limits can be linear or not. These cannot detect samples that do not correspond to any of the classes (outliers).

The aim of these discrimination methods is to find optimal boundaries between all given classes by maximizing the difference between them. A new object will always be assigned to one class, namely, to the class to which it is the most similar. This is why, these types of methods cannot be used for outlier detection, as they cannot detect samples which do not belong to any of the classes. The simplest method is the one based on the FC, the Fisher linear discriminant method. Other examples include: Discriminant partial least squares (D-PLS or PLS-DA) (30, 31) (, K-Nearest Neighbors Analysis (k-NN)32 ), Support Vector Machines (SVM) (Cristianini et al. 2000), Linear Discriminant Analysis (LDA)(Tharwat et al. 2017), Quadratic Discriminant Analysis (QDA) (Wu et al. 2022).

In this work, the SIMCA and PLSDA methods were used and compared.

1. **Materials and Methods**

2.1 Materials

Three tomato cultivars, 'Brioso,' 'Cappricia,' and 'Provine,' were used in this study. Fresh samples were harvested from different greenhouses on the 9th and 10th of May. This last day tomatoes on the vine arrived at the Phenomea Laboratory in Wageningen, Netherlands. Tomatoes without visible fungal infection were cut from the vine (2 tomatoes from the middle of a vine, 32 samples from each cultivar). The wounds at the cut end were greased with stopcock grease to prevent dehydration at the junction.

2.2. Methods

2.2.1. Data collection

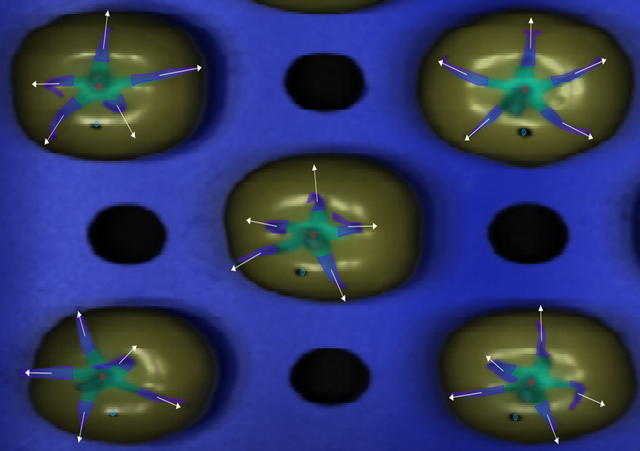
Samples were imaged in two separate groups of equal size. Hyperspectral images were recorded on day one (10th May) using a Specim FX17 NIR linescan camera with a spectral range (937.33 nm-1718 nm). Subsequently, tomatoes were stored on trays (7mm blue Forex plate (35x55cm2) with holes of 2.5 cm diameter) in controlled conditions encouraging fungal growth (20°C, in a closed sanitized box reaching 100% Relative Humidity, in a room at 60% RH, lights on during 7:00-19:00h, 15 μmol·s-1·m-2).

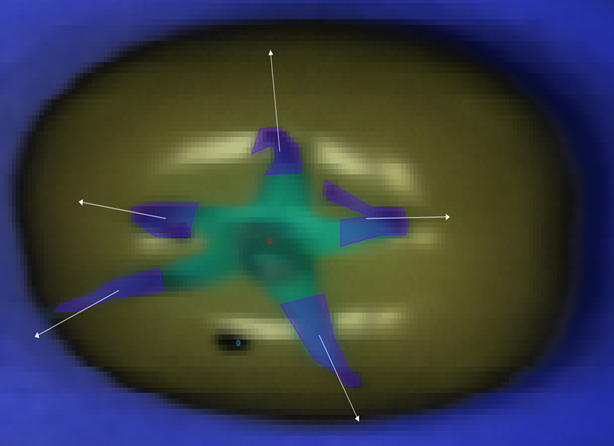
Ground truth observations were made by three experts on day three and four (12th and 13th May), comprised of severity scores from zero (no fungus) to four (severe infection). Ratings of the two days and three experts were averaged.

2.2.2. Spectra extraction from hyperspectral images

Hyperspectral images were converted to pseudo-color images. Pseudo colour images were generated after manually choosing three bands which produced visibly good contrast between sepals and the background. These images were manually annotated with a separate polygon indicating the boundary of each individual sepal. These polygons were converted to pixel masks, which indicated whether or not a pixel was included in the set of pixels belonging to the particular sepal. At sepal edges, because of blurring effects, there is some uncertainty in which pixels to include. For this annotation, we favoured keeping pixels only if they were substantially sepal containing. The spectrum of each pixel was collected and then passed to analysis.

The Darwin annotation tool from V7 labs was used to perform annotation ([https://darwin.v7labs.com](https://darwin.v7labs.com/)). Annotations were used to extract sepal pixel spectra using a custom Python image processing pipeline.





**Figure 1**: Spectra extraction from hyperspectral images. Visualization of the procedure carried out in each sepal.

2.2.3. Data analysis

A chemometric analysis was conducted with the aim of calibrating and validating models to predict the susceptibility to fungal infection in tomatoes according to their degree of disease as observed by specialists after 4 days of germination. This analysis was done using R version 4.2.1 (2022-06-23) and involved the following steps:

1. **Data Visualization**

First of all, the data set was plot to have a first appreciation of the shape of the data, observe its clarity, signal-to-noise ratio, presence of obvious outliers, baseline, etc.

1. **Data Exploration and Outlier Removal**

Exploratory analysis was carried out at sepal and variety level using Principal Component Analysis (PCA) {Fearn, 2016 #24}.

A.1. Sepal Level. PCA was applied over all the pixels for a given sepal. In order to detect outlier pixels, Mahalanobis distances were computed between the individual projection of each score value onto the model and the center of the model. The identification of outliers was determined based on a specified confidence level (0.95), indicating the probability that a data point lies within a certain range. The cutoff for Mahalanobis distances was employed as a threshold, beyond which data samples were classified as outliers. The confidence level played a crucial role in controlling the sensitivity of the outlier detection, with higher confidence levels leading to more stringent criteria for identifying outliers.

The outliers were removed, the remaining pixels averaged, and the datasets were finally reassembled according to their labels (see Table 1).

**Table 1:** Description of the dimensionality of the initial and final datasets before and after averaging spectra that belonged to the same sepal.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Dataset name/ Number of | Pixels per sepal | Sepals per tomato | Tomatoes per image | HSI | Spectra in the  initial dataset | Spectra in the averaged dataset | Variables |
| Provine | Between 119 and 90 | 5 or 6 | 16 | 2 | 16156 | 159 | 112 |
| Brioso | Between 45 and 53 | 5 or 6 | 32 | 1 | 6497 | 164 | 112 |
| Cappricia | Between 81 and 124 | 5 or 6 | 16 | 2 | 12816 | 165 | 112 |

Data exploration was carried out again by PCA in order to remove outliers at Cultivar level (in Provine, Brioso and Cappricia datasets). Score plots were created, outliers were detected visually and removed from dataset.

1. **Pretreatments to Raw Spectra**

Various pretreated forms of the original spectra were used to calibrate and validate different models, whose performances were compared with each other. These methods include: Detrend grades 1 and 2; Savitzky–Golay first and second derivatives, second polynomial degree and 9, 11, 15, 17 smoothing windows; Standard Normal Variate (SNV); and combinations of these {Jean-Michel Roger, 2022 #18}. Only the best results will be presented in this document.

1. **Data split**

Three binary-class scenarios were derived from the visual scoring explained in Data collection (2.2.1):

* Scenario 1: Score of 0 was considered healthy, and any other value was considered infected;
* Scenario 2: A score of 1 or less was considered healthy and the rest infected;
* Scenario 3: scores from two consecutive days were averaged, and a healthy class was considered when score was 0.5 or lower, otherwise the sepal was considered infected

Stratified sampling was carried out in the following way. The datasets were divided into calibration (70%) and validation (30%) sets, in a representative way for each class, randomly. The number of spectra resulting from this separation, according to each labeling scenario, can be seen in Table 2.

**Table 2:** Number of spectra in each class (Healthy: Class 1; Diseased: Class 2) when dataset was split according to different labelling scenarios (Label 1: 0/123; Label 2: 01/23 and Label 3: 0.5/123).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Cultivar | n | Label 1 | | Label 2 | | Label 3 | |
| Healthy | Diseased | Healthy | Diseased | Healthy | Diseased |
| Cappricia | 163 | 139 | 24 | 85 | 78 | 117 | 46 |
| Brioso | 153 | 145 | 8 | 78 | 75 | 126 | 27 |
| Provine | 152 | 137 | 15 | 72 | 80 | 129 | 23 |

1. **Feature Selection**

An iterative process was used to select a sparse subset of important variables in the Training set, using CovSel algorithm. Iteratively top 5 to top 39 Important Variables (ivs), (numbers chosen arbitrarily), were chosen for each pretreatment, labelling and cultivar. The selected variables were then used as input for the classification model.

Dataset with measured reflectance of only the chosen variables was saved in “CovSelTrain". The same ivs were selected from the Test Set,and saved in “CovSelTest".

1. **Calibration of PLSDA models**

The selected variables as given by CovSel were then used as input of PLSDA.

The Training set was split again, into Validation (70%) and Tuning (30%), randomly. Different models with different number of Latent Variables (LVs) were calibrated in Validation set and tested in the Tuning set. The number of LVs was selected according to the model that showed the lowest prediction error in the Tuning set.

**F) Validation of PLSDA models**

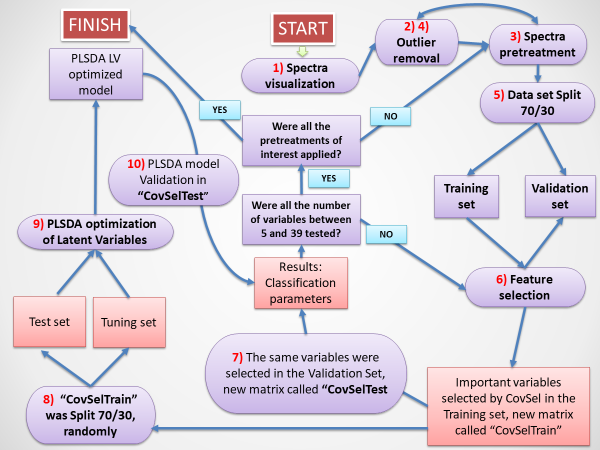
The PLSDA model, already optimized for the number of latent variables, was tested in “CovSelTest”, and classification parameters were obtained.

**G)** **Visualization and evaluation of results**

In this study, 10 parameters were used to evaluate the results: Sensitivity, Specificity, Precision, Accuracy and Balanced Accuracy, Geometric mean, F-measure, Youden index, Positive likelihood ratio, and Negative likelihood ratio. These are explained in detail in previous publications (Davide Ballabio, 2018; Akosa, 2017) and shown in Table . The evaluation should consider all of them simultaneously, because each one of them takes into account different characteristics of the general discrimination effectiveness. Google sheets were used in order to plot the results of this study.

Table : Parameters commonly used to evaluate classification models. TN: True Positives; TN: True Negatives; FN: False Negatives; FP: False Positives. Source: Akosa, 2017.

|  |  |
| --- | --- |
| Measure | Formula |
| Accuracy |  |
| Misclassification rate (1-Accuracy) |  |
| Sensitivity (or Recall) |  |
| Specificity |  |
| Precision |  |
| Balanced Accuracy (BA) |  |
| Geometric mean |  |
| Positive likelihood ratio |  |
| Negative likelihood ratio |  |
| F-measure |  |
| Youden index |  |



**Figure 1**: Diagram of the basic chemometric steps carried out in this work in order to optimize the parameters of the models.

The iterative process carried out in this work is shown in Figure 1, and can be summarized as follows:

A. Spectra visualization and outlier removal.

B. Model selection.

0. Start with a cultivar from a set of cultivars. Start with no "best model” for the cultivar.

1. Select labelling scenario (from 3 scenarios).

2. Select one pretreatment or combination of pretreatments;

3. Split Dataset.

4. Select important features.

5. Apply PLSDA and select the optimal number of latent variables (LVs).

6. Repeat Steps 4 and 5 selecting from 5 to 39 variables by CovSel.

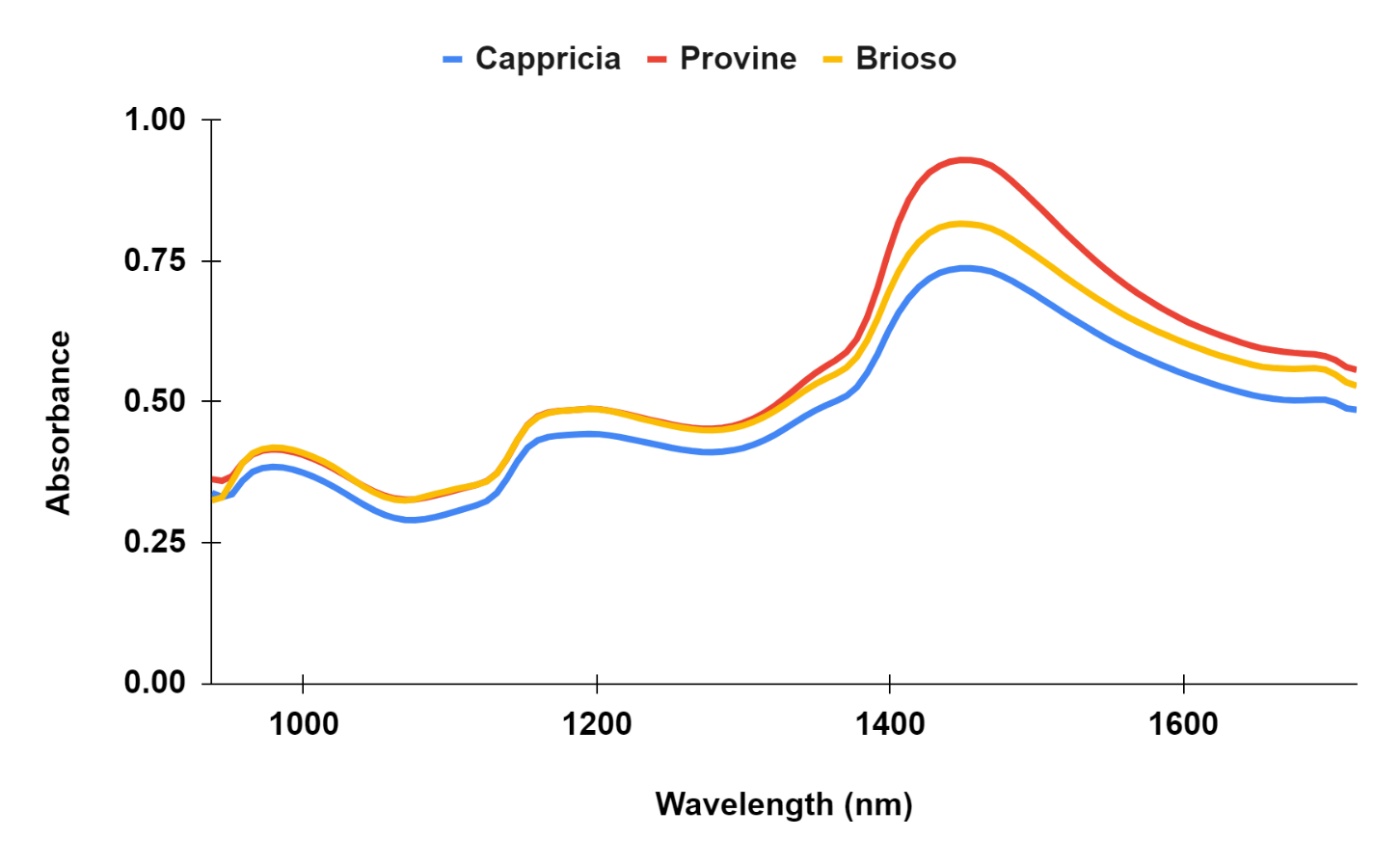
7. If a model BA is higher than the previous model, keep the current model as the “best model".

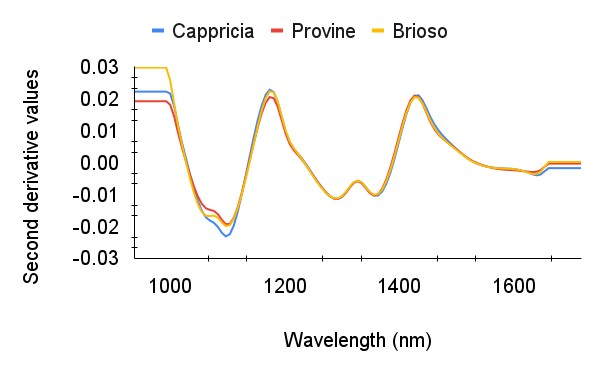
Note: Results of steps 1 to 7 will give the best model per cultivar.

C. The same process was repeated for global modeling where different scenarios of variety combinations were investigated: Cappricia+Provine, Cappricia + Brioso, Brioso + Provine and Cappricia + Provine + Brioso.

1. **Results and Discussion**

**3.1 Intravariety models**





**Figure 2**: a) Average of the raw spectra for each variety, b) Average of the spectra pretreated with SNV and second derivative (2, 17, 2).

First, the raw spectra were plot immediately after being extracted from the images. This first visualization allowed us to have a first appreciation of how the spectra looked in relation to noise and scattering effects, distortions in the baselines, signal-to-noise ratio, in addition to the presence of clear outliers. In order to understand the presence of multiplicative and/or additive effects in the spectra, their intensities were plot as a function of the average spectra (graphs not shown here). The shape of these graphs (millefeuille or cone) helped distinguish effects in the spectra. In all cases, combined effects (multiplicative and additive) were found in the analyzed spectra. Figure 2a shows the average of the raw spectra for each variety, and Figure 2b shows the average of the spectra pretreated with SNV and second derivative (2, 17, 2). As mentioned above, other pretreatments were applied and compared as well.

It is observed that raw spectra of Provine and Brioso overlapped up to approximately 1400 nm, while at longer wavelengths the average spectra were clearly differentiatedCappricia spectra showed greater intensity throughout the entire spectral range.

Three bands are observed in the pure spectra of all varieties. In the following paragraphs, tentative assignments will be mentioned along with their bibliographic sources.

The maximum intensities observed were 0.7373 (Cappicia), 0.9296 (Provine) and 0.8163 (Brioso); at 1455nm, 1447.9nm and 1447.9nm respectively, at 1455nm (6872.85cm-1) in Cappricia and at 1447.9nm (6906.08cm-1) in the other two varieties. These bands can be attributed to the symmetric and asymmetric stretching vibrations of water molecules at the first harmonic of the OH stretching vibrations of water (Muncan, 2019). More specifically, those wavelengths are included into two well-defined wavelength ranges where water shows the greatest variation of energy absorbance in response to disturbances, (Water Matrix Coordinates, “WAMACS”) (Muncan, 2019), called C8 and C9. *“WAMACS describe different conformations of water such as water dimers, trimers, superoxides, water solvation shells, etc”* (Aquaphotomics link).

The mentioned peaks in spectra can be found in WAMACS C8 and C9

C9 1458-1468nm: Water molecules with 2 hydrogen bonds (S2) (Franks 1973, Tsenkova et al. 2004)

C8 1448-1454nm: ν2 + ν3, Water solvation shell, OH-(H2O)4,5 (Cattaneo et al. 2009, Ozaki 2002).

The other peak in raw spectra was located at 1194.6 nm (8368.20cm-1) in all three varieties. According to M. Jakubíková et al. in 2016*: “the region from 8300 to 8600 cm-1 corresponds to the third overtone band of the bond CH”.* REF

In 2003, Dalimov et al. explained that tomato has approximately 11% lignin, which has carboxylic groups that distinguish it from other plants Dalimov, 2003. Moreover, these authors analyzed IR spectra of suspended tomato particles, and found typical absorption bands for lignin and carbohydrates. They assigned the 1194.6 nm wavelength to the second C–H str overtone of methyl groups, CH3-groups, as well as the lignin component of tomatoes.

However, other publications assign this band to glucose. Tanaka et al, in 2021, measured several glucose anomers in light and heavy water by NIR, and found a peak at 1195 nm in both solvents. Furthermore, Lopez et al in 2016, performed carbohydrate analysis by NIR, and assigned the same peak to the OH stretch 1st overtone of glucose. REF

Finally, the three raw spectra have a peak at 978.85nm (10241.5 cm-1). It has been assigned to the O–H stretching second overtones REF, to the*hydroxide ion (980 nm) and to the*

*Hydrogen-bonded –OH, 2nd overtone (980.4 nm). 46*

Figure 2b shows average spectra of the measured reflectance for each cultivar, after they were corrected using both the SNV and second derivative (grade=2, window width=17 and polynomial degree=2) algorithms.

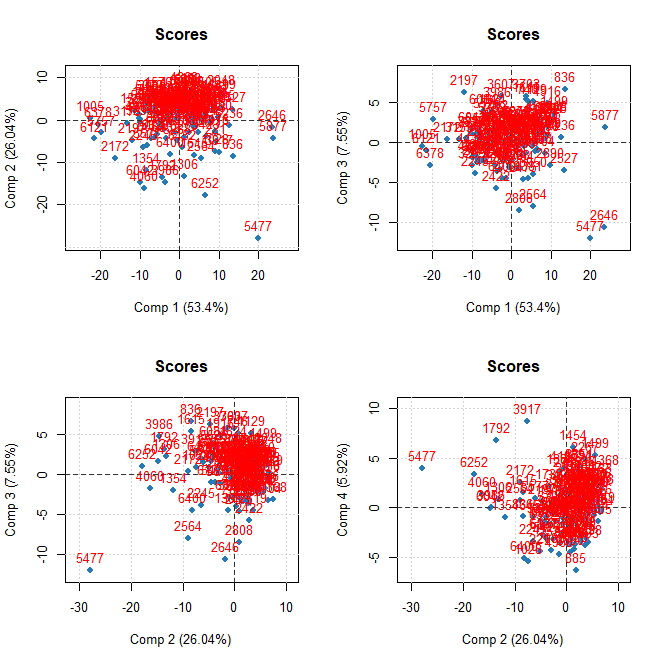
It should be mentioned that in this study, the most appropriate pretreatments were chosen according to the way in which they modify the performance of the models. Pretreatments depend on data, sometimes they can be beneficial but other times not.

In this example, SNV was used to remove the combined effect previously found in spectra. In other words,this algorithmerased both the scattering effects caused by the diffusion of photons and the measurement noise (random phenomena present throughout the entire measurement chain).The resulting spectra had media equal to zero and standard deviation equal to one.

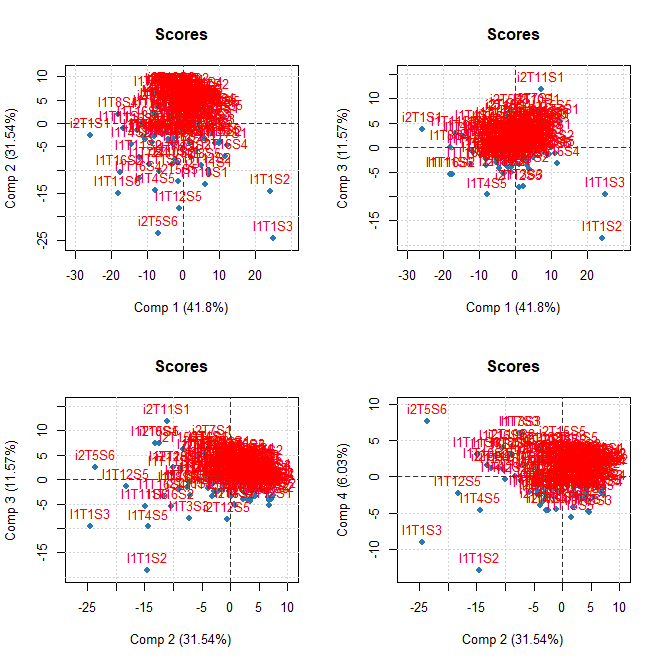
Furthermore, second derivative was used to find the exact location (center) of the shoulders in the original spectra, by deconvoluting and highlighting the peaks. As a result, we observed significantly narrower bands. Peaks appeared in the same locations as peaks in the original spectra.

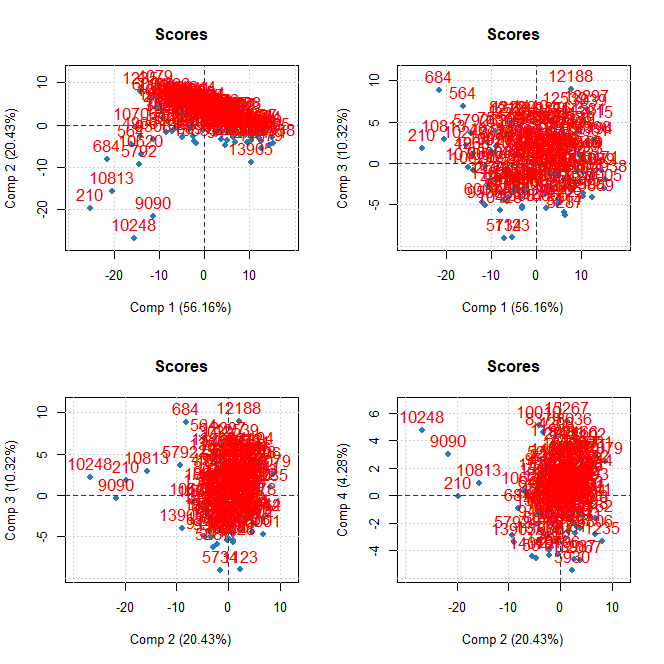
**Outlier removal using PCA**

**Brioso**



Cappricia





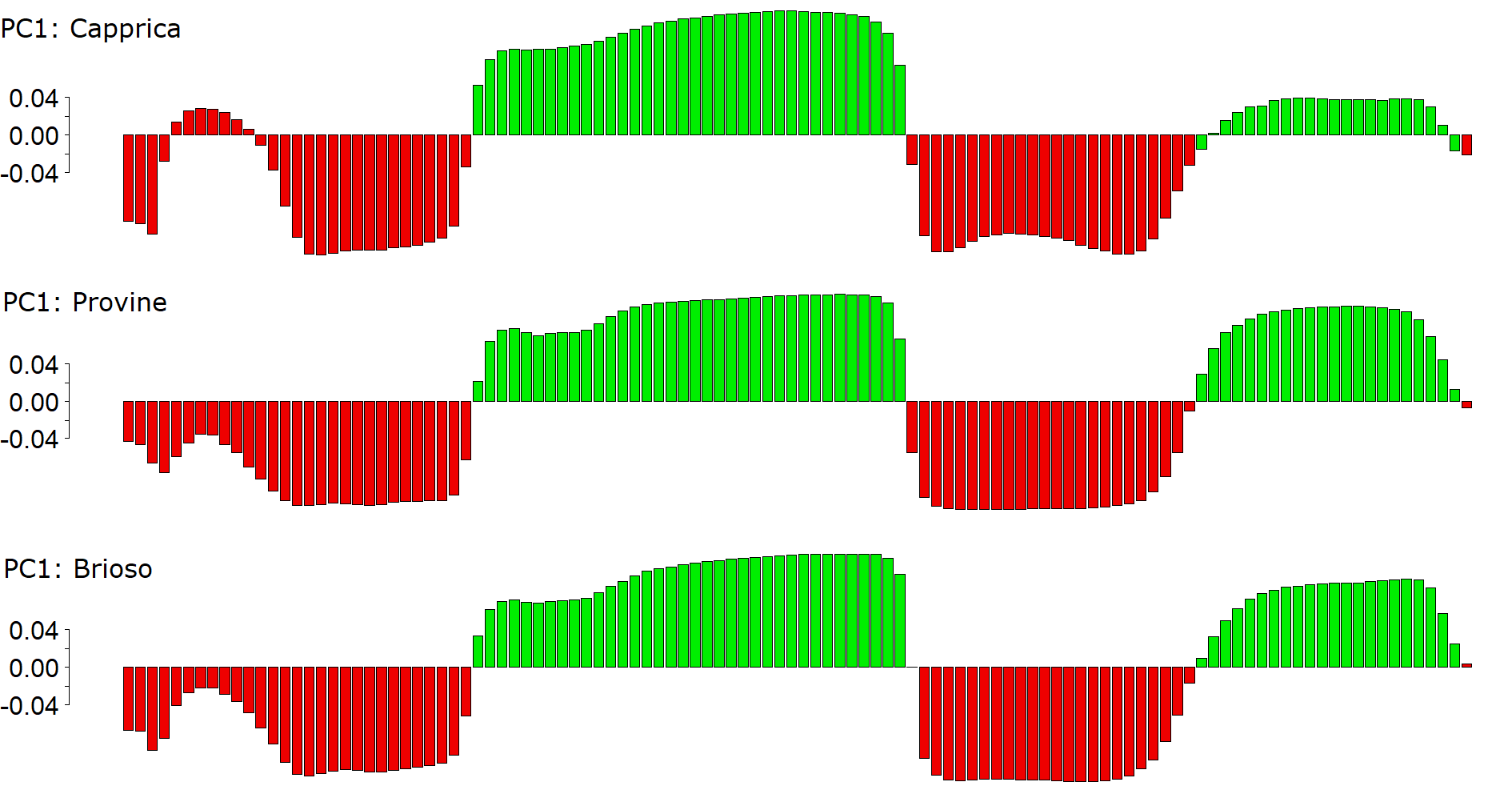
**Figure 3**: PCA score plots of a) Cappricia b) Provine c) Brioso.

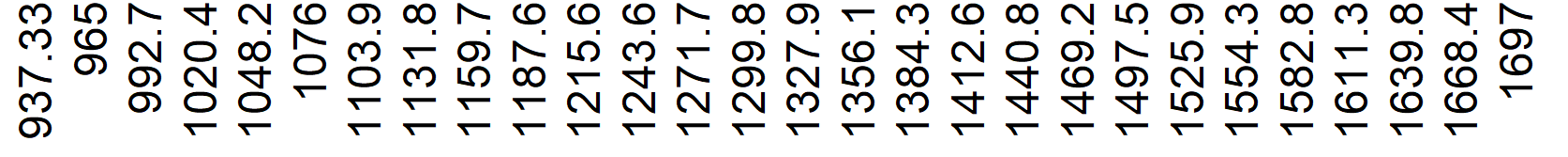
The PCA analysis was performed in this case on the pretreated spectra, first with SNV and then with the second derivative (2, 17, 2). The number of principal components to accumulate the variance explained by the model, together with the outliers detected can be seen in Table.

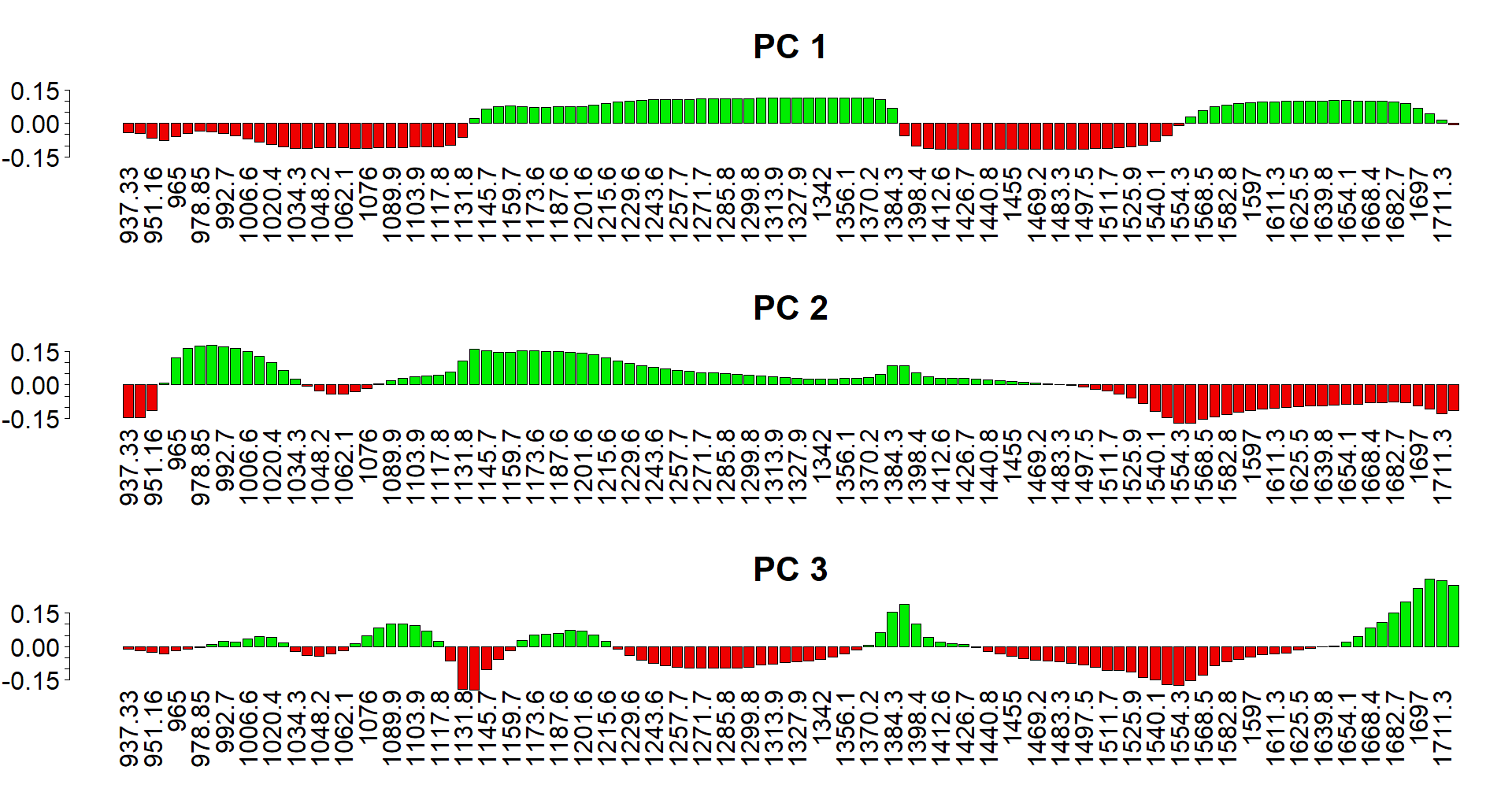
Table. Results of exploration by PCA, to detect outliers at cultivar level.

|  |  |  |  |
| --- | --- | --- | --- |
| **Cultivar** | **Number of Principal Components** | **% Variance explained by the model** | **Number of extreme outliers detected visually** |
| Brioso | 8 | 99.15 | 6 |
| Cappricia | 7 | 99.02 | 6 |
| Provine | 8 | 99.13 | 4 |

Provine







The Loadings Plots shown in Figure show that there is no notable difference between varieties in PC1 and PC2. However, PC3 is markedly different in each variety, indicating that this main component is a significant carrier of the inter-varietal distinctiveness of this pro**One-class SIMCA**

**Table. Results of One-class SIMCA on "Healthy” Class. Exp.Var: % Variance explained by the model.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cultivar** | **Exp.Var** | **TP**  **Cal** | **FP**  **Cal** | **TN**  **Cal** | **FN**  **Cal** | **Spec. Cal** | **Sens Cal.** | **Acc. Cal.** | **PC** | **Spec. Val** | **Acc. Val** |
| **Brioso** | **99.14** | **74** | **0** | **0** | **2** | **NA** | **0.974** | **0.974** | **6** | **0.157** | **0.157** |
| **Provine** | **99.83** | **72** | **0** | **0** | **4** | **NA** | **0.947** | **0.947** | **6** | **0.301** | **0.301** |
| **Cappricia** | **99.1** | **71** | **0** | **0** | **6** | **NA** | **0.922** | **0.922** | **6** | **0.270** | **0.270** |

As a first approach to the problem, a SIMCA one-class analysis was carried out, with the aim of developing a classification model in the "Healthy" class. All samples that do not correspond to this category should be rejected by the model.

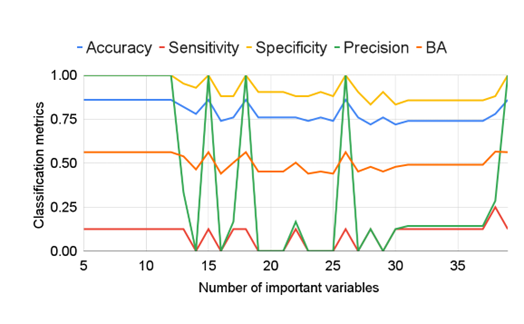
The One-Class SIMCA model gave good results for the healthy class in the three varieties (Table). However, when each trained model was validated in its corresponding diseased class, it was not able to reject samples with high specificity (Table).

This is why, SIMCA algorithm was not adequate to solve the classification problem and PLSDA algorithm was used instead.

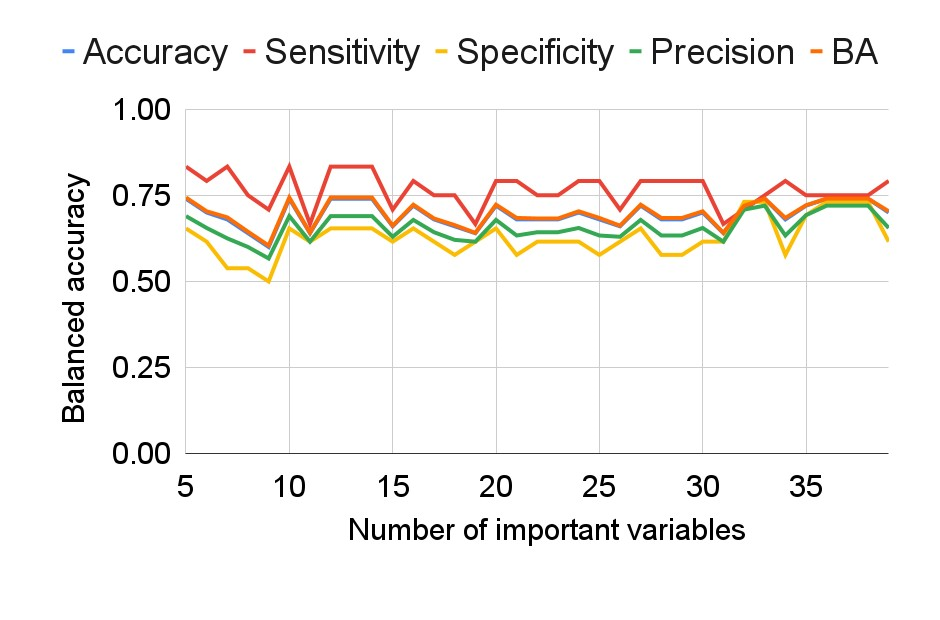
PLSDA

Brioso

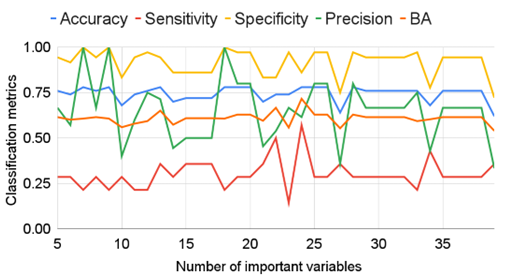
A



B



C



**Figure 2:** Cappricia classification metrics in different labelling scenarios (A: Label 1; B: Label 2; C: Label 3) according to different number of important variables as input for PLSDA.

Figure 2 (A, B and C) compares the different results obtained using different labeling scenarios between the healthy and the diseased classes for Cappricia cultivar. As can be observed, the precision metric behaved erratically when Scenario 1 was selected. This metric showed high values when less than 13 variables were chosen, but then decreased abruptly with 14 variables; and increased again when 15 variables were chosen. This counterintuitive behavior was due to the fact that the precision metric took into account false positives in the denominator, which changed abruptly with different splits. In other words, the behavior of the precision metric showed that the data were not uniformly distributed in both classes, when Scenarios 1 and 3 were chosen.

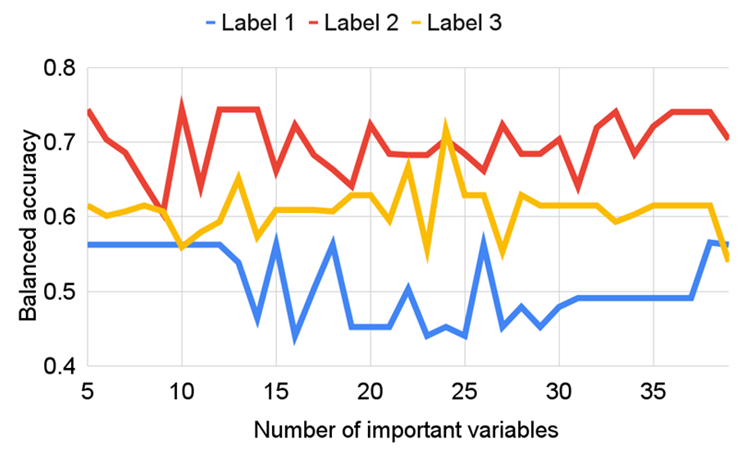
Another indicator of class balancing was the correlation between the accuracy (blue line) and the balanced accuracy (BA) (orange line). When the classes were balanced, these metrics were almost identical, and their lines overlapped as we could see in Scenario 2 (Figure B). On the other hand, in Figures 1. A and 1. C, a clear separation was observed between them, the accuracy was higher than BA.

As a rule, high BA values showed that the model performances were good for both classes. On the other hand, high accuracy metric showed that the model performed well in general. When using Scenarios 1 and 3, this was the case for the majority classes only.

It should be mentioned that there are several ways to solve data imbalance. One of them is with oversampling (adding samples from the least represented class); or with undersampling (deleting samples from the majority class). In the first case, the risk of overfitting increases, since during cross-validation, the same samples that are in the model can be used to validate it; in the second case, important information is removed from the model.

A one-class classification could also have been used, where all samples similar to the samples of one class are considered, and the others discarded by the model. However, these models are always less specific, and in the case of the present study, they showed poorer classification parameters.

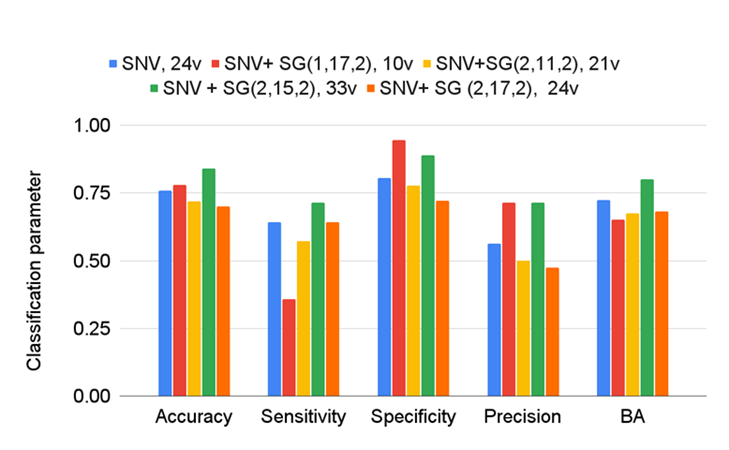
Due to these reasons, Scenario 2 was chosen to calibrate and validate the models. No addition or removal of samples was made, except for the aforementioned outliers.



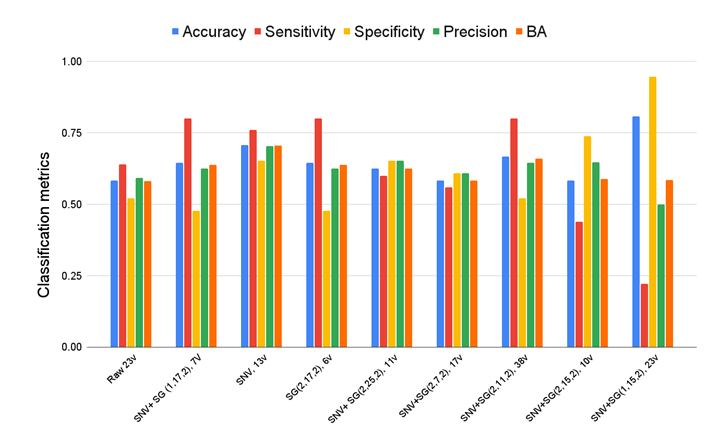
**Figure 3:** Comparison of Balanced Accuracy in different labelling scenarios according to different number of important variables as input for PLSDA.

The relationship between the BA of the models versus the number of important variables (ivs) chosen by CovSel can be seen in Figure 3, for the different Scenarios 1, 2 and 3. Once again, we can see that Scenario 2 was the best option, because it showed higher BA values.

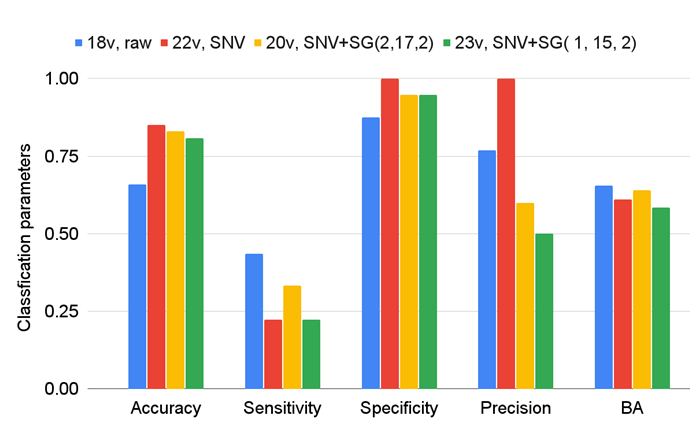
Cappricia



Provine



Brioso



**Figure 4:** Comparison of Balanced Accuracy in different labelling scenarios according to different number of important variables as input for PLSDA.

Figure 4 shows the results of the optimized models for Cappricia, Provine and Brioso, choosing Scenario 2. The following parameters generated the optimal models:

Cappricia: SNV + Second Derivative (2, 15, 2) and 33 ivs

Provine: SNV and 13 ivs

Brioso: Raw spectra and 18 ivs

Cappricia: Optimal model was found with pretreated data (SNV+ SG (2,15,2)) and 33 most important variables. Provine: Optimal model was found with SNV pretreated data and 13 most ivs Brioso: Optimal model was found with raw data and 18 ivs.

**3.2. Intervariety models**

**Table 5:** PLSDA classification model calibrated in Cappricia and validated in Brioso and Provine. Pretreatment used: SNV+ SG(2, 17, 2); all spectral range and 3 Latent Variables (LVs).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data set** | **Real/**  **predicted** | **Healthy** | **Diseased** | **NA** | **SEN** | **SPE** | **PRE** | **AC** | **BA** |
| **Calibration**  **Cappriccia** | **Healthy** | **66** | **11** | **0** | **0.86** | **0.55** | **0.63** | **0.70** | **0.71** |
| **Diseased** | **38** | **46** | **0** | **0.55** | **0.86** | **0.81** | **0.70** | **0.71** |
| **Validation**  **Brioso** | **Healthy** | **67** | **7** | **0** | **0.91** | **0.22** | **0.54** | **0.56** | **0.57** |
| **Diseased** | **57** | **16** | **0** | **0.22** | **0.91** | **0.70** | **0.56** | **0.57** |
| **Validation**  **Provine** | **Healthy** | **80** | **0** | **0** | **1** | **0.03** | **0.54** | **0.54** | **0.52** |
| **Diseased** | **69** | **2** | **0** | **0.03** | **1** | **1** | **0.54** | **0.52** |

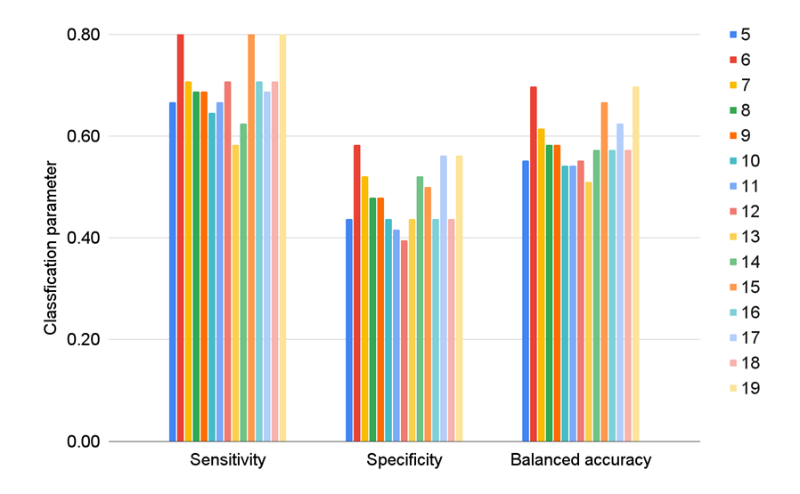
The intervariety models presented high sensitivity for one class (0.91; 1) but not for the others (0.22, 0.03) (Table 5) in the validation sets.

**3.3. Global models**

**Table 6:** PLSDA Classification results of a global model, calibrated and validated with Cappricia and Provine.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data set** | **Real/**  **predicted** | **Healthy** | **Diseased** | **NA** | **Sensitivity** | **Specificity** | **Precision** | **BA** |
| **Calibration** | **Healthy** | **62** | **47** | **0** | **0.57** | **0.74** | **0.68** | **0.66** |
| **Diseased** | **29** | **81** | **0** | **0.74** | **0.57** | **0.63** | **0.66** |
| **Validation** | **Healthy** | **23** | **25** | **0** | **0.48** | **0.71** | **0.62** | **0.60** |
| **Diseased** | **14** | **34** | **0** | **0.71** | **0.48** | **0.58** | **0.60** |

Table 6 shows PLSDA classification results of a global model, calibrated and validated with cultivars Cappricia and Provine. In this model, spectra were pretreated with SNV, and then with second derivative (2, 17, 2). Then, 19 important variables were chosen by the CovSel algorithm. Finally, the PLSDA model was trained with the calibration data, and 17 latent variables were chosen. This relatively high number can be understood as being due to the complexity of adding two different varieties in one model.

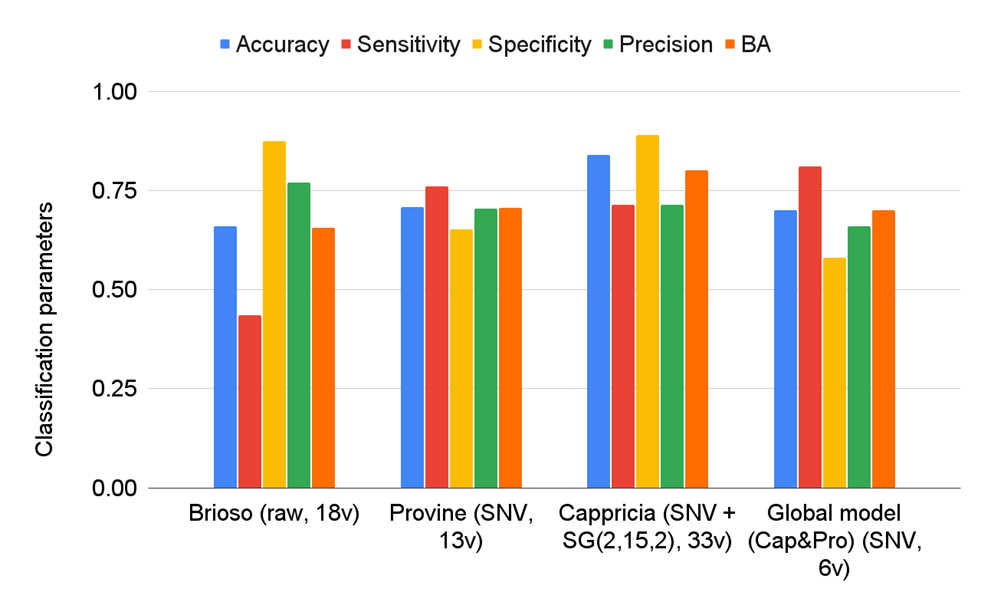


**Figure 5:** Classification parameters of the validation sets for the global model trained with Cappricia and Provine according to the number of important variables as input for PLSDA. Data were pretreated with SNV + SG (2,17,2).

In Figure 5, the values of Sensitivity, Specificity and Balanced Accuracy are shown for an increasing number of variables between 5 and 19. As can be seen, choosing only six variables produced similar results in terms of balanced accuracy as choosing 19.

**Table 7:** PLSDA Classification results of all the optimal models in this study.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter/**  **Model** | **Cappricia**  **Raw, 15v**  **Label 3** | **Cappricia**  **SNV + SG(2,15,2), 33v**  **Label 2** | **Provine**  **Raw, 14v**  **Label 3** | **Provine**  **SNV, 13v**  **Label 2** | **Brioso**  **Raw, 18v**  **Label 2** | **Global model**  **SNV, (Cap+Pro)**  **6v**  **Label 2** |
| Accuracy | 0.83 | 0.84 | 0.71 | 0.71 | 0.66 | 0.70 |
| Sensitivity or recall | 0.89 | 0.71 | 0.08 | 0.76 | 0.43 | 0.81 |
| Specificity | 0.64 | 0.89 | 0.97 | 0.65 | 0.88 | 0.58 |
| Precision | 0.89 | 0.71 | 0.50 | 0.70 | 0.77 | 0.66 |
| Balanced accuracy | 0.77 | 0.80 | 0.52 | 0.71 | 0.65 | 0.70 |
| Geometric mean | 0.75 | 0.79 | 0.27 | 0.70 | 0.62 | 0.69 |
| F-measure | 0.89 | 0.71 | 0.14 | 0.73 | 0.55 | 0.73 |
| Youden’s Index | 0.53 | 0.60 | 0.05 | 0.41 | 0.31 | 0.39 |
| Positive likelihood ratio | 2.47 | 6.45 | 2.67 | 2.17 | 3.58 | 1.93 |
| Negative  likelihood ratio | 0.17 | 0.32 | 0.95 | 0.37 | 0.65 | 0.33 |



**Figure 7:** Classification parameters of the validation sets for the optimal models validated in this study.

Table 7 and Figure 8, show PLSDA modeling results of all the optimal models in this study. BA was comparable to traditional accuracy in all models created with Scenario 2, showing that the classifier performed equally well on either class.

Accurate predictions for healthy sepals were as follows: Cappricia (0.71), Provine (0.76), Global model (0.81). Similarly, for diseased sepals correctly classified as such: Cappricia (0.89), Provine (0.65), Global model (0.58).

Moreover, good performances on both positive and negative classes were found in the Cappricia Intravariety model. High positive likelihood ratio of 6.45 (above 1: increased evidence for disease-free) for the Healthy class; Low negative likelihood ratio of 0.32 (increased evidence for disease) for the Infected class.

The intervariety models calibrated in Cappricia showed high sensitivity in class one (0.91) and low specificity in class two; this was consistent in prediction for Brioso (0.03) and Provine (0.11).

For two-class classification, the geometric mean was calculated as the square root of the product of specificity and sensitivity. As a rule, If one of the classes cannot be recognized by the model, this parameter tends to zero. REF. This parameter showed this behavior, when its values were less than 0.5. This is was observed in the case of sample classification of the Provine variety using scenario 3: although the specificity of this model was high, the sensitivity was very low (0.08), and the GM was 0.28. In all other cases, this parameter was greater than 0.5 showing that the models were able to recognize both classes.

The added value of the F-score parameter lies in the fact that it shows a combination of precision and recall.

In the case that the classes are balanced, the parameter F1 can be ignored, the best indicator being the accuracy (or the misclassification rate).

However, in the event that you have a highly imbalanced data set but both classes are important, it is recommended to choose a model that presents high F1 values in both classes. If otherwise only one class is important, it is useful to choose a high F1 value only for that class. Poner el valor de F1 en las dos clases

1. **Conclusion**

This work was carried out with the objective of developing a method to detect the susceptibility of freshly harvested tomatoes to the presence of fungi, in non-destructive ways, and before the disease can be observed visually.

For this, hyperspectral images of the samples were measured, and models were developed based on their relationships with ground truth data.

The models can be divided into three general categories: those calibrated and validated using a single variety (intravariety), and finally those calibrated and validated with several varieties together (global models). In all cases, the best results were found using scenario 2 as a reference.

Within the first category, the optimal model was created with the Cappricia variety: Balanced accuracy= 0.80; Sensitivity= 0.71 and Specificity= 0.89.

As for the global models, the optimal models were calibrated using Cappricia and Provine together: Balanced accuracy=0.70, Sensitivity=0.81; Specificity=0.58.

The results from this research suggest the conclusion that discrimination between more susceptible and less susceptible samples is feasible under controlled conditions.

**Acknowledgements**

The authors would like to thank the EU commission for funding obtained through the ANTARES Horizon 2020 project (grant 664387).

**Conflicts of interest**

The authors declare no conflicts of interest.

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