1. **Introduction**

Tomato (Solanum lycopersicum L.) is a "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.2 Market quality grade (e.g. color, form, size), firmness and shelf life, whereas health benefits rely on the nutritional value as well as on the absence of pathogenic hazards or contaminants.(2-5)

The portion of tomatoes that go to waste after the harvesting stage can reach 42% worldwide.(6) 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*.(7) Pathogenic fungi can infect and spread to many different parts of a tomato plant, including the stem, calyx and skin of the fruit.(8) 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.(9) This negatively affects the value of the fruit and may lead to extra food loss and waste.(9, 10)

The timely identification of disease has the potential to avert losses since prompt actions can be implemented to mitigate further damages (e.g. adapt packing strategies).(9) 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.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 individual tomatoes or even batches from growth to harvest and later post-harvest handling and logistics is highly difficult.

Some tomatoes are more susceptible to infection and growth of spores while others are not.(9, 11) 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.(12) Moreover, tomato samples can be tested for mycotoxins, as a high level of these compounds is caused by fungal infection.(13) Some detection solutions are, for instance, chromatography coupled with detector methods, electrochemical biosensors technology and immunological techniques such as such enzyme-linked immunosorbent assay, dipsticks and flow-through membranes. (14-17) 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.(13)

Although these analytical methods are specific and accurate, they have several disadvantages. First, 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, they evaluate what is happening to the fruit exactly at the moment of the measurement. 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 the fruits 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 and post-harvest management.

Infrared spectroscopy can provide a possible solution to this problem. Paul Skolik et al, in 2019, have studied diseased progression in whole tomatoes using Attenuated total reflection coupled with Fourier-transform infrared spectroscopy (“AFT-FTIR”) and have highlighted that plant-pathogen interaction can be identified through alteration in the spectra fingerprint.(18)

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. in 2021, accurately classified 97.5% of healthy fruit and 100% of decayed fruit using spectral imaging.(19)

With similar motivation as the current article, in a previous study, Brdar et al. investigated ensemble machine learning methods, for early detection of sepals’ sensitivity to fungal infections on one tomato cultivar (Brioso).(11) 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, select features 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 recently harvested tomatoes. Therefore, this is the objective of our research.

The methodology used for our 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.(20, 21)

2. Background

2.1. Feature selection

In constructing chemometric models, it is recommended to carefully select variables (which refers to wavelengths or wavenumbers in spectroscopy).(22) 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.(23) 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 three main challenges: NIR spectra are of high dimension the variables are highly correlated and obscured by the presence of overlapping absorbances, harmonics, and combination bands. Moreover, spectra are often complicated by light scattering and other physical effects.

Two or more variables, such as v1, v2, v3 and v4, are described as multicollinear when:

v₁ = α₁ + α₂v₂ + α₃v₃ + α₄v₄ Eq(1)

The effect that this phenomenon will have on the efficient use of the algorithms will be described later (See Section 2.3 Classification methods). https://medium.com/@raj5287/effects-of-multi-collinearity-in-logistic-regression-svm-rf-af6766d91f1b

According to Roger et al. in 2011, it is therefore important to limit the outcome space, since this is directly proportional to the number of variables in a dataset. (24) It is also important to note that some variables are only 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 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.

The feature selection methods can be classified as Filters, Embedded and Wrapper, according to the interconnection with the problem to be solved. (22)

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.(22)

When embedded methods are used, variable selection is carried out at the same time as learning by cross-validation performance. (25)

On the other hand, wrapper methods iteratively choose a subset of features, train the models, and choose then the best combination of variables. They use the model's error rate on a holdout set to score feature subsets. (22)

In previous works, embedded methods (Random Forests) were used to solve a similar research problem. (11) This is why, in the present study, a wrapper method (the CovSel algorithm) was chosen to compare results and to evaluate the performance of a different approach.(26)

When using the CovSel algorithm, the significance of each wavelength is assessed by calculating the covariance between each variable and the Y values (responses). (27)

Initially, the X and Y matrices undergo centering, wherein the mean value is subtracted from each individual value within each dataset, consequently setting the mean value of each dataset to 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. (24)

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 one 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, 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.(28)

In 2009, James Burger found that bad pixels exhibit significantly different spectra compared to their neighbors.(29) These abnormal pixels were classified 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. (30)

Moreover, some pixels are always noisy, while others are noisy only sporadically; some may show a “non-linear response to light intensity” while some others behave randomly.(31)In any case, these abnormal pixels exhibit distinct behaviour compared to the rest and thus should be removed. In this work, they were detected in an unsupervised manner (see Section 2.2.3.b 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 creates 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); Potential function methods (POTFUN) and Unequal variance methods (UNEQ).(32-35)

Unlike probabilistic methods (such as POTFUN and UNEQ), which rely on the assumption of a specific distribution or a probabilistic structure, non- probabilistic methods (such as SIMCA) have the advantage of being able to deal with complex data. Non-probability methods can be flexible and robust when probabilistic predictions are hard to make or when data is very heterogeneous or not well modeled by well-known distributions. They can also be easier to implement and easier to understand, which makes them useful in environments where interpretation and simplification are important.  (36)

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 boundaries 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 Fisher linear discriminant method. Other examples include Discriminant partial least squares (D-PLS or PLS-DA), K-Nearest Neighbors Analysis (k-NN), Support Vector Machines (SVM), Linear Discriminant Analysis (LDA) and Quadratic Discriminant Analysis (QDA).(37-40)

PLSDA is superior to other algorithms such as k-NN, SVM, and LDA/QDA in high-dimensional datasets because it can address high dimensionality and multicollinearity problems by selecting latent variables based on cross-validation. Furthermore, when a data collection contains more variables than samples, LDA and QDA cannot be employed alone; instead, a dimensionality reduction method must be used first. Unlike k-NN, which performs worse as features increase, PLSDA guarantees accurate discrimination by preventing the collapse of similarity assumptions among observations. Additionally, SVM is susceptible to abrupt changes in weight vectors caused by multicollinearity. (41-43)

As a result, in this study, the SIMCA and PLSDA methods were selected from each category (class modeling versus simple discrimination) for comparison purposes.

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 2022. On the 10th May 2022, the 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).(11) 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 per sepal by three experts on day three and four (12th and 13th May), which 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, which 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 individual sepals (Figure 1). These polygons were converted to pixel masks, which indicated whether a pixel was included in the set of pixels belonging to a particular sepal. At sepal edges, because of blurring effects, there is some level of uncertainty with respect to 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 used for further analysis.

The Darwin annotation tool from V7 labs was used to perform annotations.(44) Annotations were used to extract sepal pixel spectra using a custom Python image processing pipeline.(45)

[insert Figure 1.]

**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 Statistical Software (v4.3.0; R Core Team 2021) with caret, rchemo and prospectr packages, and involved the following steps:(46-49)

1. Data visualization

Firstly, spectra were plotted to have a first appreciation of the shape of the data, observe their 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).(50)

PCA was applied over all the pixels for a given sepal. 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.

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

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

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

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

1. Pretreatments on raw spectra

Different models were calibrated and validated using various pretreated forms of the original spectra, and their performances were compared. 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.(51-53) Only the best results are presented in this document.

d) Data split

Three binary-class scenarios were derived from the visual expert scoring described 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 samples were considered healthy when the score was 0.5 or lower, otherwise the sepal was considered infected.

Stratified sampling was carried out in the following way. Each dataset was divided into calibration (70%) and validation (30%) sets, in a representative way for each class, randomly. This means that the 70/30 ratio was respected in both classes. Table 2 shows the number of samples belonging to each class according to each Labeling Scenario, in the complete datasets.

**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 | 77 | 86 | 117 | 46 |
| Brioso | 153 | 145 | 8 | 74 | 77 | 126 | 27 |
| Provine | 152 | 137 | 15 | 83 | 74 | 129 | 23 |

e) Feature selection

An iterative process was used to select a sparse subset of important variables from the Training set, using CovSel algorithm.(27, 54) 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, and saved in a matrix called "CovSelTrain” The same ivs were selected from the Test Set, and saved in “CovSelTest".

f) Calibration and validation of PLSDA models

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

Table 3 shows how the original number of samples belonging to each class was then divided into the Training (70%), Test (30%) sets, consistently for both classes (the details of the calculation can also be seen). Then, the Training sets were randomly divided into Calibration (70%) and Validation (30%) (Table 3).

**Table 3:** The number of samples in each set, after dividing the original datasets into Train set (70%), Test set (30%), randomly. The Train set was split again into Calibration set (70%) and Validation set (30%).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Provine | | Brioso | | Cappricia | | Global model | |
| Class | Healthy | Diseased | Healthy | Diseased | Healthy | Diseased | Healthy | Diseased |
| Nb. of samples | 83 | 74 | 74 | 77 | 77 | 86 | 160 | 160 |
| Training | 83\*0.7=58 | 74\*0.7=52 | 74\*0.7=52 | 77\*0.7=54 | 77\*0.7=54 | 86\*0.7=60 | 160\*0.7=112 | 160\*0.7=112 |
| Test | 83\*0.3=25 | 74\*0.3=22 | 74\*0.3=22 | 77\*0.3=23 | 77\*0.3=23 | 86\*0.3=26 | 160\*0.3=48 | 160\*0.3=48 |
| Total Training | 58+52=110 | | 52+54=106 | | 54+60=114 | | 112+112=224 | |
| Total Test | 25+22=47 | | 22+23=45 | | 23+26=49 | | 48+48=96 | |
| Calibration | 110\*0.7=77 | | 106\*0.7=74 | | 114\*0.7=80 | | 224\*0.7=157 | |
| Validation | 110\*0.3=33 | | 106\*0.3=32 | | 114\*0.3=34 | | 224\*0.3=67 | |

All models calibrated in the Calibration set must be tested (validated) later, in the Validation set. A model might yield exceptional and highly accurate outcomes when applied to the Calibration set. However, if overfitting occurs, the same model may produce poor results when evaluated using the Validation set. In other words, an overfitted model fits perfectly well the Calibration set, but cannot be generalized for efficient use in new, unknown samples.

To avoid this, the optimal number of latent variables must be chosen, according to the error observed in the Validation set, when the model is tested on independent samples, which were not used during its calibration.

The prediction error in the Calibration set can always decrease, carrying a risk of overfitting. Instead, the prediction error in the Validation set decreases up to an optimal number of LVs, after which it increases. At this inflection point the optimal number of LVs that should be chosen for the model can be known. If a greater number of LVs is chosen, the model will have a risk of overfitting.

In other words, for each latent variable number, a prediction error value is obtained in the Validation set. It is necessary to know all the prediction error values that correspond to all the different numbers of Lvs and choose the one that entails the smallest prediction error, in the Validation set.

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

g) Evaluation of results

In this study, 10 parameters were used to evaluate the results: Sensitivity, Specificity, Precision, Accuracy and Balanced Accuracy (BA), Geometric mean, F-measure, Youden index, Positive likelihood ratio, and Negative likelihood ratio. These are explained in detail in previous publications and shown in Table 4.(55, 56) The final evaluation considered all of them simultaneously, because each one of them takes into account different characteristics of the general discrimination effectiveness.

**Table 4:** 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 |  |

[insert Figure 2]

**Figure 2**: 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 2, 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 Interpretation of raw spectra

A

[insert Figure 3A]

B

[insert Figure 3B]

**Figure 3**. Raw (A) and SNV and second derivative (2, 17, 2) spectra (B) for each variety.

It can be observed that raw spectra of Provine and Brioso overlapped until approximately 1400 nm, while at longer wavelengths the average spectra were clearly differentiated.

Three bands are observed in the pure and pretreated spectra of all varieties (Figures 3A and 3B). 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 (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.(57) 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”), called C8 and C9. *“WAMACS describe different conformations of water such as water dimers, trimers, superoxides, water solvation shells, etc”* .(57, 58)

*C9* *1458-1468nm:* *Water molecules with 2 hydrogen bonds (S2)*

*C8* *1448-1454nm:*  *ν2 + ν3, Water solvation shell, OH-(H2O)4,5.(58, 59)*

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”.(60)*

In 2003, Dalimov et al. concluded that tomato has approximately 11% lignin with carboxylic groups that distinguish it from other plants.40 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 stretching overtones 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.(61) Furthermore, Lopez et al in 2016, performed carbohydrate analysis by NIR, and assigned the same peak to the OH stretch 1st overtone of glucose.(62)

Finally, the three raw spectra have a peak at 978.85nm (10241.5 cm-1). It has been assigned in literature to the O–H stretching second overtones, to the hydroxide ion (980 nm) and to the hydrogen-bonded –OH, 2nd overtone (980.4 nm).(63, 64)

3.2. Intravariety models

3.2.1. Pretreatments and Exploratory Analysis

First, the raw spectra were plotted 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. To understand the presence of multiplicative and/or additive effects in the spectra, their intensities were plotted 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 3 (on the left) shows the average of the raw spectra for each variety, and Figure 3 (on the right) 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 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.

In this example, SNV was used to remove 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, the second derivative allowed to find the exact location (center) of the shoulders in the original spectra, by deconvoluting and highlighting the peaks. As a result, significantly narrower bands were observed. The peaks appeared in the same locations as the peaks in the original spectra.

[insert Figure 4]

**Figure 4**: PCA score plots of Cappricia cultivar.

The PCA analysis was performed in this case on the pretreated spectra, first with SNV and then with the second derivative (2, 17, 2), in all three varieties. Scores plots for Cappricia are shown in Figure 4. The number of principal components to accumulate the variance explained by each model, together with the number of outliers detected can be seen in Table 5.

**Table 5.** 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 |

[insert Figure 5]

**Figure 5**: PC 1 Loading plots of cultivars Cappricia, Provine and Brioso.

[insert Figure 6]

**Figure 6**: PC 2 Loading plots of cultivars Cappricia, Provine and Brioso.

As previously mentioned, we have conducted principal component analysis individually for each variety, as well as collectively for all varieties combined. Opting for the former approach affords us a more nuanced understanding of the intrinsic variability inherent to each distinct variety. Our focus will now center on elucidating the observed patterns within the loadings of the initial three principal components (PC1, PC2, and PC3). It's worth noting that while our analysis predominantly relies on these three components, a more comprehensive examination necessitates a greater number of components to adequately account for the variance observed within each dataset. For instance, in the case of Cappricia, 99.02% of the variance is explicated by 7 principal components, Provine exhibits 99.13% variance explained by 8 PCs, and Brioso manifests 99.15% variance explained by 8 PCs.

These loadings represent the correlation between the original variables (wavelengths) and the principal components. Loadings of greater magnitude indicate stronger correlations between the variables (wavelengths) and the principal components. The sign of the loadings indicates the direction of the correlation. Positive loadings indicate a positive correlation between the wavelength and the principal component, while negative loadings indicate a negative correlation.

Despite some variability, PC1 and PC2 loadings across the three varieties (Figures 5 and 6), reveal common trends across varieties, particularly in regions where the loadings exhibit pronounced peaks or troughs. These common trends suggest consistent patterns in how the variables are related to the underlying structure captured by the principal components. However, the magnitude of the loadings varies across cultivars. In PC1, for example, in Brioso, the absorbance values range from -0.123 to 0.154, in Cappricia from -0.079 to 0.197, and in Provine from -0.147 to 0.165. These variations indicate differences in the intensity of spectral features across the cultivars, reflecting distinct chemical compositions or structural characteristics present in the samples analyzed. Higher magnitude loadings indicate stronger correlations between the variables (wavelengths) and the principal components.

When it comes to the Loadings Plot of PC1 (Figure), In the Brioso dataset, prominent peaks are evident at wavelengths 1006.6 nm, 1062.1 nm, and 1540.1 nm, featuring absorbance values of -0.0717, 0.0842, and -0.0549, respectively, while corresponding troughs appear at wavelengths 1027.4 nm, 1201.6 nm, and 1654.1 nm, with absorbance values of -0.0808, 0.0104, and -0.1115, respectively. Similarly, in the Cappricia dataset, peaks align at the same wavelengths with slightly different absorbance values (-0.0965, 0.1002, and -0.0465, respectively), accompanied by troughs at 1027.4 nm, 1201.6 nm, and 1654.1 nm, with absorbance values of -0.0889, -0.0465, and -0.1115, respectively. Likewise, the Provine dataset showcases peaks at the aforementioned wavelengths, exhibiting absorbance values of 0.0661, -0.0878, and 0.0511, respectively, while troughs emerge at wavelengths 1027.4 nm, 1201.6 nm, and 1654.1 nm, with absorbance values of 0.1041, -0.0465, and 0.1097, respectively. While Brioso demonstrates stability with consistent absorbance values across wavelengths, Provine and Cappricia exhibit more dynamic behavior with larger variations in absorbance values. Cappricia shows moderate fluctuations and trends, while Provine displays more pronounced changes and clearer trends in absorbance across wavelengths.

When it comes to the loadings in PC2 (Figure 6), the important wavelengths, or peak locations, are found at approximately 1034.3, 1041.3, 1048.2, 1055.2, 1062.1, and 1069.1 nanometers. These specific wavelengths correspond to distinct molecular or chemical features present in the samples of Brioso, Cappricia, and Provine. The consistency of these peak locations across all three varieties suggests that these wavelengths are crucial for characterizing and distinguishing between the chemical compositions or properties of the samples. However, similar to PC1, there are differences in the magnitudes in the absorbance values across cultivars. Magnitude comparison of absorbance values at key wavelengths reveals that Brioso generally exhibits higher absorbance values compared to Cappricia and Provine. For instance, at 1034.3 nm, Brioso shows an absorbance of 0.0176, while Cappricia and Provine both have 0.0421. This trend persists across other wavelengths such as 1041.3 nm (Brioso: 0.0454, Cappricia: -0.0027, Provine: -0.0027) and 1055.2 nm (Brioso: 0.0724, Cappricia: -0.0638, Provine: -0.0638). However, Cappricia and Provine exhibit similar absorbance values at these wavelengths, suggesting a higher degree of similarity between them in PC2 compared to Brioso.

[insert Figure 7]

**Figure 7:** PC 3 Loading plots of cultivars Cappricia, Provine and Brioso.

When it comes to the loadings in PC3 (Figure 7), the comparison of absorbance values among the three varieties reveals distinct spectral patterns and magnitudes across various wavelengths. Brioso generally exhibits higher absorbance values compared to Cappricia and Provine, particularly evident in wavelengths around 1020 nm to 1070 nm, where Brioso shows peaks with magnitudes ranging from 0.0806 to 0.2178, while Cappricia and Provine have significantly lower values, with peaks ranging from -0.0052 to 0.1395. Moreover, Brioso tends to have more pronounced peaks at specific wavelengths, such as 1124.8 nm and 1131.8 nm, where its absorbance values sharply rise to 0.2021 and 0.2071, respectively, compared to Cappricia and Provine. However, Provine exhibits distinctive peaks at longer wavelengths beyond 1400 nm, with absorbance values ranging from -0.1284 to 0.2089, indicating unique molecular features not as prominent in Brioso and Cappricia. Additionally, Cappricia demonstrates relatively consistent absorbance values across most wavelengths, with peaks ranging from -0.1393 to 0.2327, suggesting a more balanced distribution of molecular features compared to the other varieties.

Between 1055 and 1412 nm, Brioso and Provine exhibit notable differences in their absorbance patterns. Brioso generally displays higher absorbance values compared to Provine across this wavelength range. Specifically, in the region around 1124.8 nm, Brioso shows a distinct peak with an absorbance value of 0.2021, while Provine's absorbance remains comparatively lower, around -0.1790. Similarly, at wavelengths like 1250.7 nm and 1271.7 nm, Brioso demonstrates peaks with absorbance values of 0.1939 and 0.2279, respectively, whereas Provine's absorbance values are notably lower, ranging from -0.0501 to -0.1033. This trend continues throughout the range, with Brioso consistently exhibiting higher absorbance values and more pronounced peaks compared to Provine. These differences suggest variations in the molecular composition or concentration of certain chemical features between Brioso and Provine within this spectral region, indicating potential distinctions in their biochemical properties or applications.

After 1300 nm, Brioso and Cappricia show differences in their absorbance patterns, indicating potential variations in their chemical compositions or molecular structures. Specifically, between 1300 and 1400 nm, Brioso exhibits relatively higher absorbance values compared to Cappricia. For instance, at around 1313.9 nm, Brioso displays a peak with an absorbance value of 0.0597, while Cappricia's absorbance in the same region is lower, around 0.0024. Similarly, at 1377.3 nm, Brioso shows a notable peak with an absorbance value of -0.1217, whereas Cappricia's absorbance remains comparatively lower, around -0.1125. Between 1300 and 1600 nm, Brioso consistently shows higher absorbance values and more pronounced peaks compared to Cappricia.

3.2.2. Classification

Different results obtained using different labeling scenarios between the healthy and the diseased classes for Cappricia cultivar were compared (plots not shown). As a result, 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 and the balanced accuracy. When the classes were balanced, these metrics were almost identical, and their lines overlapped as we could see in Scenario 2. On the other hand, a clear separation was observed between them, the accuracy was higher than the BA (graph not shown).

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, given the existing dataset balance. When using Scenarios 1 and 3, this was the case only for the majority classes.

It should be mentioned that there are several ways to solve data imbalance. One of them is oversampling (adding samples from the least represented class); another one is undersampling (deleting samples from the majority class). In the first case, poor implementation risks overfitting, 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 can be removed from the model.

A one-class classification could also have been used, where all samples similar to the samples of one class are included, 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 metrics.

For example, a One-class SIMCA analysis on the 'Healthy' classes revealed high variance explained by the models, with values of 99.14% for Brioso, 99.83% for Provine, and 99.1% for Cappricia. The model gave good results for the healthy class in the three varieties. In the calibration set (Cal), Brioso achieved a True Positive (TP) of 74, a Specificity and Sensitivity (Sens Cal.) of 0.974, while Provine showed a TP of 72, Spec. Cal and Sens Cal of 0.947 and Cappricia exhibited a TP of 71, Spec. Cal and Sens Cal of 0.922. However, when the trained models were validated in their corresponding diseased classes, they were not able to reject samples with high specificity. In the validation set (Val), Brioso demonstrated an Accuracy (Acc. Val.) of 0.157, Provine had an Acc. Val. of 0.301, and Cappricia achieved an Acc. Val. of 0.270. These validation metrics offer insights into the robustness of the model across different cultivars (Table 6).

**Table 6.** 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 |

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.

[insert Figure 8]

**Figure 8:** Comparison of Balanced Accuracy in different labelling scenarios according to different number of important variables as input for PLSDA.

The relationship between BA and ivs can be seen in Figure 8, 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.

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

3.3. Global models

3.3.1. Classification

Table 7 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 in 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.

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

**Table 8:** 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 |

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

For two-class classification, the geometric mean (GM) was calculated as the square root of the product of specificity and sensitivity (Table 3). As a rule, if one of the classes cannot be recognized by the model, GM tends to zero. (65) This parameter showed this behavior, when its values were less than 0.5. This 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.

**4) Conclusion**

This work was carried out with the objective of developing a method to predict the susceptibility of freshly harvested tomatoes to the presence of fungi, in a non-destructive way, before the disease can be observed visually. To this aim, 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 two general categories: those calibrated and validated using a single variety (intravariety), and those calibrated and validated with several varieties together (global models). In both 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= 0.84, 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 sepals is feasible under controlled conditions.

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