Thesis of the PhD Dissertation

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Development of innovative rapid methods for the qualification and authentication of fruits and fruit products

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1. INTRODUCTION

Food consumption trends have evolved significantly over the recent years, particularly in the context of health consciousness, environmental awareness, and the impacts of the COVID-19 pandemic. These trends can be particularly tracked in the consumption of fruits and fruit products, which have seen fluctuations in demand influenced by various socio-economic influences, consumer preferences and public health messages. Ensuring the quality expected by buyers and consumers poses a significant challenge. Fruits to reach store shelves in the form, degree of processing, and quality we seek, they have to go through a very complex journey, through the food chain as we know it. The first challenges arise right in the orchards, consider the mild winters, frosty springs, drought-stricken summers, and the multitude of pests. Then comes the critical question of when to begin harvesting. How long, where, and under what conditions should fruits be ripened and/or stored, so that the industry can process them in so many of ways.

In fruit production and quality control practice, the commonly applied assessments are often based on empirical, and often destructive methods. By empirical, we refer to when producers determine the start of harvest based on traits such as how easily the fruit detaches from the stalk, how easily the fruit flesh cracks, or how sweet is the fruit after tasting, that are inherently subjective. A more objective approach involves the instrumental measurement of fruit weight, colour, firmness, and sugar content. These are targeted techniques, designed to assess a single characteristic at a time, and on their own, they are insufficient for capturing the full spectrum of quality differences. Ensuring the authenticity and traceability of food products is almost unimaginable without the use of digital solutions, and innovative rapid methods allow us to conduct non-destructive and even contactless analyses directly on-site. During such analyses, hundreds or even thousands of data at a time can be collected, forming the "fingerprint" of a given sample. Based on these fingerprints, paired with reference characteristics and chemometric modelling, the non-destructive qualification of previously unknown samples become possible. We increasingly rely on tuneable digital solutions that can be trained to address a wide range of questions, that are driven by chemometric modelling.

The objectives of this doctoral research were realised in cooperation with Agricolae Ltd., a company based in the Szatmár region (Hungary), renowned for its fruit production. The focus of the thesis is on the widespread application of spectroscopy-based techniques in fruit production and quality assessment, specifically at certain critical points within the fruit supply chain. Within the framework of the collaboration, fruit species and varieties have been included that hold significant economic importance both within and beyond national borders. Additionally, determining their physiological state (e.g., ripeness, microbiological contamination) or detecting specific manipulations of products made from them that poses particular challenges.

Near infrared (NIR) spectroscopy is one of the advanced correlative analytical methods that are widely used in routine laboratory or industrial monitoring systems, utilising the wavelength range of 780 to 2500 nm (Manley 2014). The proliferation of miniaturisation techniques and their application in image processing technologies, like hyperspectral imaging, has made it possible to study complex biological systems in an intact way. During our investigations, we addressed the question of the efficiency of NIR spectroscopy and hyperspectral imaging coupled with chemometric modelling for the determination of fruit ripeness and thus the determination of harvest time, the efficiency of detecting inadequate fruit storage, *Monilinia* causing brown rot as well as fruit juice enrichment.

2. OBJECTIVES

The aim of the thesis was the application and development of *state-of-the-art* correlative analytical methods for non-destructive characterization of fruits and fruit products (namely fruit juices). In this doctoral research three main objectives were established, which, with the corresponding tasks, are as follows.

I. **Determining the applicability of NIR spectroscopy to determine the ripeness of various stone fruits.** Achieving this aim was supported by the following tasks:
   - Spectral tracing of maturation and ripening processes with hand-held spectrometer,
   - Model development for the classification of fruits according to their ripeness levels,
   - Model development for the prediction of certain quality traits of fruits.

II. **Determining the applicability of NIR spectroscopy and hyperspectral image processing for the detection of brown rot caused by *Monilinia* on different stone fruits.** Achieving this aim was supported by the following tasks:
   - Monitoring changes during refrigerated or room temperature storage of intact and damaged fruits contaminated with different concentrations of *Monilinia* conidium suspension,
   - Conducting investigations with hand-held NIR spectrometer and hyperspectral imaging,
   - Model development for pinpointing the effect of different storage conditions,
   - Model development for the detection of fruits contaminated with *Monilinia* in various ways and to various degrees,
   - Development of sorting models for the early detection of fruits suspected for brown rot.

III. **Determining the applicability of NIR spectroscopy for the detection of enrichment/manipulation of fruit juices with plant extracts.** Achieving this aim was supported by the following tasks:
   - Spectral analyses of fruit juices enriched with plant extracts in various combination and concentration using hand-held and benchtop NIR spectrometers,
   - Model development for the classification according to the type of extract, and dosed concentration,
   - Model development for the prediction of added extract content.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Fruit samples analysed during the ripeness assessment studies

The non-destructive determination of ripeness, and thus the optimal harvest time of various stone fruits was carried out by analysing fruit samples from Szabolcs-Szatmár-Bereg county. During the summer months of 2021, seasonal sweet cherries ("Bigarreau Burlat", "Valery Chkalov"), sour cherries ("Kántorjánosi", "Újfehértói") and plums ("Elena", "Stanley") were examined. According to the ripening period of the fruits, the harvesting took place in June, July and August. Harvesting was done in two distinct phases, one week apart per fruit species. After each harvest, the fruits were promptly transported to the to the Institute of Food Science and Technology (IFST), Hungarian University of Agriculture and Life Sciences (MATE). The incoming batches exhibited significant variability; their ripeness ranged from unsuitable for consumption to fully ripe. After sample arrival, the stem removal and sorting of the fruit was started as soon as possible to avoid any undesirable perishing processes. The preliminary classification of the fruits according to their presumed ripeness was based on the overall visible colour shade differences, varying from the very green to deep red or purple. The sample sets into which a relatively large number of fruits were initially sorted were further divided into subsets. To facilitate the interpretation of research outcomes, the pre-classified sample sets were then grouped into larger ripeness clusters. The resulting sample counts are the following.

- **Sweet cherry varieties:**

  Bigarreau Burlat (BB): 26 pre-classified sample groups × 5 replicates = 130 samples

  Valery Chkalov (VC): 21 pre-classified sample groups × 5 replicates = 105 samples
- **Sour cherry varieties:**

  Kántorjánosi (KJ): 20 pre-classified sample groups × 5 replicates = 100 samples

  Újfehértói (UF): 21 pre-classified sample groups × 5 replicates = 105 samples
- **Plum varieties:**

  Elena (EL): 20 pre-classified sample groups × 5 replicates = 100 samples

  Stanley (ST): 20 pre-classified sample groups × 5 replicates = 100 samples.

3.1.2. Fruit samples analysed during the Monilinia detection studies

For the non-destructive study of the processes involved in brown rot of stone fruits, different sour cherry and plum varieties were included in the research. The harvest and examination of the fruit varieties were conducted at different times. The experiments on "Érdi bőtermő" (EB) and "Újfehértói" (UF) sour cherries, as well as "Topend" (TD) plums, were conducted in 2021. The experiments on "Topend plus" (TP) plums were carried out in 2022. The pre-selected experimental fruits were uniform in ripeness, colour, and free from any visible damage for each variety.

Isolation of *Monilinia* species causing brown rot was performed from the surface of various fruits (e.g., sour cherry, plum). After several attempts, it was possible to successfully isolate and propagate *M. fructigena* on culture media. The fruits intended for conidium production were disinfected with ethanol solution (70% V/V), wounded using a sterile lancet needle, and agar discs overgrown with mycelium from a pure pathogen culture were placed into the wounds. To produce conidia, sour cherries, plums and apples were infected and were stored in a Fitotron growth chamber on 21°C with a 12-hour light cycle. After approximately seven days, conidia formed on the surface of the inoculated

fruits were collected, then conidial suspensions were prepared (~10^5 conidium/ mL) and diluted in accordance with Horváthné Petróczy (2009).

Before starting the sample actual preparation, the stems of the sour cherries and plums were removed and their surface was gently cleaned with precision wipes soaked in ethanol solution (70% V/V). This step was necessary because in these series of experiments, we focused solely on the detectability of *Monilinia* and aimed to minimize any potential spoilage processes caused by unwanted other microbes.

After cleaning, a 5 mm incision was made with the tip of a disinfected knife on the surface of a portion of the fruits into which 20 µL of undiluted (~10^2 conidium/ µL) or diluted *M. fructigena* conidial suspension was pipetted. These fruits constituted the "$\text{Injury}_{\text{inf}}$" samples. For another portion of the fruits, 20 µL of the suspensions was applied without making any incisions, constituting the "$\text{Intact}_{\text{inf}}$" samples. The remaining fruits were not inoculated, serving as the "$\text{Intact}_{\text{con}}$" and "$\text{Injury}_{\text{con}}$" samples, the latter was prepared only for *Topend plus* plums.

The prepared fruits were subjected to seven days of refrigerated (around 5 °C) or room temperature (above 20 °C) storage under controlled conditions. In the case of room temperature storage, the storage environment was adjusted to the room temperature typical at the time of fruit preparation. The decaying processes were investigated using two distinct non-destructive analytical techniques, NIR spectroscopy and hyperspectral imaging, for which two identically prepared sample sets were established. For each sample group, five replicates were prepared, resulting in the following sample sizes:

- **Sour cherry varieties:**

  Érdi bőtermő (EB)

  $$((\text{Intact}_{\text{con}} + 4\ \text{Injury}_{\text{inf}} + 4\ \text{Intact}_{\text{inf}}) \times 2 \text{ storage conditions}) \times 5 \text{ replicates} =$$
  $$90 \text{ sour cherry samples}$$

  Újfehértói (UF)

  $$((\text{Intact}_{\text{con}} + 4\ \text{Injury}_{\text{inf}} + 4\ \text{Intact}_{\text{inf}}) \times 2 \text{ storage conditions}) \times 5 \text{ replicates} =$$
  $$90 \text{ sour cherry samples}$$

- **Plum varieties:**

  Topend (TD)

  $$((\text{Intact}_{\text{con}} + 3\ \text{Injury}_{\text{inf}} + 3\ \text{Intact}_{\text{inf}}) \times 2 \text{ storage conditions}) \times 5 \text{ replicates} =$$
  $$70 \text{ plum samples}$$

  Topend plus (TP)

  $$((\text{Intact}_{\text{con}} + \text{Injury}_{\text{con}} + 3\ \text{Injury}_{\text{inf}} + 3\ \text{Intact}_{\text{inf}}) \times 2 \text{ storage conditions}) \times 5 \text{ replicates} =$$
  $$80 \text{ plum samples}$$

3.1.3. Samples analysed during the fruit juice enrichment studies

For these experiments, juices prepared from sour cherry and plum concentrates were analysed to minimise the risk of unwanted and unknown product manipulation in our samples. Based on the initial concentration of fruit juice concentrates (approx. 65% brix), pure stock juices were prepared of about 20% brix in SSC by dilution with distilled water. Cranberry (CBE), grape seed (GSE) or pomegranate extracts (PGE) were added to the juices at six concentration levels so that their total concentration ranged between 0.5 and 2.5 g/100 mL. These formed the simple blends, which were then mixed in

equal proportions by concentration level to produce binary and ternary juice blends. In these cases, the aim was also to ensure that the total extract content of the resulting juice blends remained between 0.5 and 2.5 g/100 mL. During the preparation of the fruit juice blends, three parallel juice samples were prepared per sample group. For the pure sour cherry and plum juices five replicate samples were prepared. These samples represented the 0 g/100 mL concentration level. The sample sizes for each fruit juice are shown in Eq. 1, 2. The prepared fruit juice samples were pipetted into 15 mL autoclavable centrifuge tubes, heat-treated (85 °C, 60 s), cooled and refrigerated until non-destructive analytical methods.

(5 pure juices) + (3 extracts × 6 conc. level × 3 replicates) + (3 binary mixtures × 6 conc. level × 3 replicates) + (1 tertiary mixtures × 6 conc. level × 3 replicates) = **131 blends of sour cherry juice** (1)

(5 pure juices) + (3 extracts × 6 conc. level × 3 replicates) + (3 binary mixtures × 6 conc. level × 3 replicates) + (1 tertiary mixtures × 6 conc. level × 3 replicates) = **131 blends of plum juice** (2)

3.2. Applied methods

3.2.1. Reference methods applied in the fruit ripeness assessment studies

Determination of colour characteristics of stone fruits

The colour characteristics of stone fruits of varying ripeness were determined using a ColorLite sph850 spectrophotometer (ColorLite GmbH, Germany). Following calibration, the instrument was used to measure the colour attributes, such as lightness (L*), green-red (a*), and blue-yellow (b*), on both the immature and mature sides of the fruits. All five parallel samples of the pre-classified sample groups were measured respectively in a randomised measurement order. Three consecutive measurements were taken for each colour attribute. The averages of these measurements were used in the subsequent data analysis.

Determination of dry matter content of stone fruits

To determine the dry matter content (DMC) of stone fruits of varying ripeness, the flesh of the fruits was chopped by pre-classified sample sets. Approximately 10 g of chopped fruits were measured in and gently dried at 70 °C in an air-conditioned airing cupboard (Memmert, Schwabach, Germany) to constant weight. Due to the high sugar content of the samples, gentle drying was necessary to avoid damaging them. The dry matter content was calculated as the ratio of the dry weight to the initial weight (Schuck, Dolivet, and Jeantet 2012). Three measurements were conducted for each sample group, respectively, and the averages of these were used for subsequent data analysis.

Determination of total acidity of stone fruits

To determine the total acidity (TA) of stone fruits of varying ripeness, the previously chopped fruits were pureed with a kitchen stick blender (Philips, Amsterdam, Netherlands). Approximately 10 g of fruit puree was measured in and the acid concent was determined by potentiometric titration with 0.1 mol/ dm^3 sodium hydroxide solution (pH 8.2) in the presence of phenolphthalein indicator. During the measurement, a Hanna HI2209 benchtop pH meter was utilised (Hanna Instruments, Smithfield, USA). The total acidity was calculated and expressed as mg/ g (fresh weight) malic acid (Tyl and Sadler 2017). Three measurements were conducted for each pre-classified sample group, respectively, and the averages of these were used in the subsequent data analysis.

Determination of total soluble solid content of stone fruits

To determine the total soluble solid content (SSC) of stone fruits of varying ripeness, the previously blended fruits were measured in a tube of 50 mL, centrifuged at 6000 rpm for 20 min (Micro 22R Hettich, Germany), and a few drops of the supernatant juice were measured with a digital refractometer (Pocket PAL-1, ATAGO, Tokyo, Japan). Following calibration with distilled water, the device provided results of fruit juice soluble solid content expressed in % brix (Chockchaisawasdee et al. 2016). Three measurements were conducted for each pre-classified sample group, respectively, and the averages of these was used in the subsequent data analysis.

Determination total anthocyanin content of stone fruits

To determine total anthocyanin content (TAC) of stone fruits of different ripeness, the previously prepared supernatant juice was measured in and analysed using the pH differential method (Lao and Giusti 2016; Lee et al. 2005, 2016), as described in the studies by Fodor et al. (2022, 2023). These measurements required the use of a pH meter (Hanna Instruments, Smithfield, USA) and UV-Vis spectrophotometer (Thermo Electronic UV-Vis 2.02, Thermo Fisher Scientific, Waltham, MA, USA). The results were expressed in cyanidin-3-glucoside equivalent in mg/ L. Three measurements were performed for each pre-classified sample group, and the averages of these measurements was used in the subsequent data analysis.

3.2.2. Near infrared spectroscopy for the fruit quality assessment studies

Application of hand-held NIR spectrometer for the determination of stone fruit ripeness

To non-destructively model the harvest maturity of various stone fruits, near-infrared (NIR) spectroscopy was applied. The investigations were conducted with a hand-held reflection-based NIR spectrometer (NIR-S-G1, InnoSpectra Co., Hsinchu, Taiwan). The device enables contact measurement with internal illumination in a total of 256 spectral bands in the 900-1700 nm wavelength range using the Hadamard method. For the fruits, spectra were recorded on both the immature and mature sides of the five parallel samples of the pre-classified sample sets. At each measurement position, three consecutive spectrum recording was performed. The fruits were scanned in a randomised measurement order.

Application of hand-held NIR spectrometer for the detection of *Monilinia* on stone fruits

The hand-held NIR instrument and setup used for the non-destructive analysis of sour cherries and plums infected with *M. fructigena* and stored under different conditions were consistent with the configuration described above. On sour cherries, spectra were recorded along the horizontal axis of the fruits; on plums, they were captured along the vertical axis, with three measurement points per fruit. The second measurement point was always the point of inoculation on the fruit. At each measurement position, three consecutive spectrum recording was performed. After each measurement position, the contact surface of the device was disinfected with alcohol-soaked precision wipes to prevent cross-contamination. Spectral data collection was performed daily throughout the seven-day long storage. The fruits were scanned in a randomised measurement order.

Application of hand-held NIR spectrometer for the control of enriched fruit juices

For the examination of plant extract-enriched fruit juice blends, a hand-held MicroNIR spectrometer (Viavi, Scottsdale, USA) was employed in transflectance measurement arrangement. The device enables contact measurement with internal illumination in a total of 125 spectral bands in the 900-1700 nm wavelength range. The spectra of the juices were recorded in a cylindrical glass cuvette with

a reflective surface that provided a layer thickness of 0.5 mm. To compensate the initially large number of fruit juice blends, the pure fruit juices were scanned multiple times. Specifically, the five replicate samples of sour cherry and plum juice were each scanned three times in total. Three consecutive spectra were recorded during each sample loading. Between each sample measurement, the cuvette was thoroughly cleaned with distilled water and ethanol (70% V/V), then dried and rinsed with the upcoming juice sample to prevent cross-contamination. The juices were scanned in a randomized measurement order. These measurements were conducted at the Institute of Analytical Chemistry and Radiochemistry (University of Innsbruck, Austria).

Application of benchtop NIR spectrometer for the control of enriched fruit juices

The spectral properties of the fruit juices were also examined in a transmission measurement arrangement. A modular Fourier transform spectrometer was utilized for this purpose (NIRFlex N-500, Büchi Labortechnik AG, Flawil, Switzerland), acquiring data in a total of 1178 spectral bands within the wavelength range of 1000-1890 nm. Similarly, as detailed above, the samples were analysed in a randomized order with three consecutive scanning using a glass cuvette with a path length of 1 mm. The cuvette was cleaned as described above with additional drying with compressed purified air to remove as much of the excess cleaning moisture from the cuvette as possible. The cuvette was also rinsed with the upcoming juice sample. These measurements were conducted at the Institute of Analytical Chemistry and Radiochemistry (University of Innsbruck, Austria).

3.2.3. Hyperspectral imaging for the detection of Monilinia contamination on stone fruits

For the non-destructive and contactless analysis of sour cherries and plums infected with *M. fructigena* and stored under various conditions, a desktop Headwall Photonics XEVA-1648 XC134 hyperspectral imaging (HSI) system was utilised (Specim spectrograph, Xenics InGaAs 14-bit sensor, 256 × 320 px spectral and spatial resolution). This system allowed the NIR spectral and spatial characterization of fruits at the same time. The instrument operated in a push-broom configuration, capturing a total of 155 spectral bands in the 900-1700 nm wavelength range, with a spectral resolution of 5 nm and a spatial resolution of 0.475 mm per pixel. The measuring system was operated using the department-developed Argus software (Firtha 2011). Randomised HSI was performed daily on the plum samples and on six days in total for the sour cherry samples during their seven-day long storage. The segmentation of fruit-related pixels from the data recorded in a hypercube with the HSI system was performed using a department-developed HyperGrab hyperspectral image processing software (GillaySoft, Budapest, Hungary). The software allowed to extract the average absorbance values from nine surface areas per measurement at a time.

3.3. Evaluation of the research results

The organization, evaluation, and illustration of the data were performed using Miscrosoft Excel 365 (Microsoft Corporation, Redmond, Washington, USA), Origin Pro 2018 (OriginLab Corp. Northampton, MA, USA), R-project (version 3.6.3) and the “aquap2” package (Pollner and Kovacs 2016). This section summarises the methods used to evaluate the study results.

3.3.1. Evaluation of results obtained with reference measurements

Reference measurements are only available on the samples analysed during the ripeness determination studies. The colour (L*, a*, b*) and compositional characteristics (i.e., DMC, SSC, TA, TAC) obtained from the pre-classified sample sets were averaged according to ripeness clusters.

3.3.2. Evaluation of results obtained using correlative analytical methods

Pre-processing of the spectral data

The spectra obtained through NIR spectroscopy and hyperspectral image processing were first truncated to the wavelength ranges intended for evaluation, followed by the removal of outliers and subsequent multivariate statistical analyses. In experiments where the efficiency of different instruments was compared (e.g., benchtop and hand-held NIR devices), the evaluations were performed within the same wavelength ranges, and in neither case were the spectra averaged. For noise reduction in the spectra, Savitzky-Golay smoothing (second-order polynomial) was applied (Savitzky and Golay 1951), along with other spectral pre-processing methods (e.g., scatter correction, detrending, derivatives) to optimise statistical modelling.

Principal component analysis of the spectral data

Principal component analysis (PCA) was applied to compress the highly correlated NIR spectral data into variables (principal components, PCs) that no longer correlate with each other. Additionally, this method allowed for the identification of outliers by detecting data points that fell outside the 95% confidence interval. This function of the PCA was only used for the evaluation of experimental results aimed at monitoring storage and detectability of *Monilinia*-caused decay on stone fruits.

PCA modelling was also used as a preliminary pattern exploration on smoothed (Savitzky-Golay smoothing with 2nd order polynomial, 21 data points; "sgol-2-21-0") and multiplicative scatter-corrected (msc) data. Furthermore, the results revealed how individual wavelengths correlate with the PCs, which were illustrated on loading plots. PCA models were built on the whole dataset by fruit variety, respectively, to recognise patterns in fruit ripeness, in *Monilinia*-infected fruit handling and storage, or in total added extract content in enriched fruit juices.

Soft independent modelling of class analogies

Soft independent modelling of class analogies (SIMCA) was only used to evaluate the experimental results of *Monilinia* detection studies. The method was applied alongside the PCA results, except that modelling was performed on data recorded at the beginning (day 1), middle (day 4) and end of storage (day 7), also after smoothing and msc pre-treatments. This supervised classification method helped to better understand the similarities and differences between the sample groups which for these evaluations were the different treatments (mode of inoculation and storage condition together).

SIMCA models the multivariate space formed by a given sample group and calculates whether a given observation belongs to a specific group based on the interclass distances and the importance of variables (i.e., wavelengths). This approach also gives the discriminating power of the variables, significantly contributing to group differentiation and thereby facilitating the identification of absorbance bands associated with spectral differences (Wold and Sjostrom 1977).

Linear discriminant analysis of the spectral data

Linear discriminant analysis (LDA) was performed as a supervised classification method to discriminate and classify samples according to various classification variables. In our specific application, principal component scores served as the input values for the LDA models. The optimal number of principal components (NrPCs) used in the modelling process was determined by an R-based algorithm that collected and compared the LDA model calibration and validation accuracies up to a predefined 20 NrPCs, using three-fold cross-validation. The NrPCs that yielded the smallest

difference between model calibration and validation accuracy, as well as the highest validation accuracy, was selected for the actual modelling.

The PCA-LDA models were built on data filtered to certain data sets, pre-processed then further optimised for NrPCs. The models were tested using leave-one-sample replicate-out (LOSO) validation meaning that model construction was done by omitting data of one parallel sample from the available sample sets at a time. During testing, the data of the previously omitted samples were projected into the constructed PCA-LDA model. Model building and testing were completed cyclically until all data for each sample were included at least once in the modelling. The classification results (%) obtained during the construction and testing of the models are summarised in so-called confusion tables. In addition, this method also assisted in identifying absorbance bands which contributed greatly to the differentiation between sample groups.

In the ripeness studies, models classifying by ripeness levels were first built by fruit variety, and then separately on the data obtained on the more mature and immature sides of the fruits. This was performed to explore the influence of the location of spectrum acquisition on classification accuracy.

In the experiments related to *Monilinia* detectability, classification models were built by treatment group based on spectral pre-processing- and NrPC-optimised data recorded the day after sample inoculation to determine the detectability of the initial conidial contamination on the fruit surface. For this modelling, the classification was done for conidium contamination levels of about 0.1, 1, 10, 100 conidia/ µL. In addition, the spectral trend of samples that were found to undergo *Monilinia*-induced spoilage during storage was also modelled. This only concerned fruits that were infected through injury and stored at room temperature ("~ 20 °C $Injury_{inf}$"). After specifically determining on which storage day the fruits exhibited visible signs of *Monilinia* infection (marked as "0 day"), data covering ± 2-day interval relative to data for "day 0" were included in this modelling.

PCA-LDA was also applied for the qualitative classification of fruit juice blends enriched with plant extracts in various concentration. Models were built to detect the type of extracts administered in simple, binary, or ternary combinations, as well as to group them based on the total added extract concentration.

Partial least squares regression on the spectral data

Partial least squares regression (PLSR) was applied to predict quantitative compositional attributes based on the spectral data. PLSR models were developed individually for each fruit variety using filtered, spectral pre-processing- and latent variable- (NrLV) optimised data. Model validation was conducted through leave-one-sample replicate-out (LOSO) validation, as described for LDA, ensuring robust assessment of predictive performance. The accuracy and reliability of the models were quantified by the coefficient of determination (R^2) and the root mean square error (RMSE). The model fitting accuracies obtained during the model building (C) and testing (CV) by predicted attribute are arranged in summarising tables. In addition, the regression vectors were also obtained as partial results, which provide information on the degree to which each variable is correlated with the actually predicted parameter.

In the ripeness studies, PLSR models were employed to non-destructively predict certain quality traits (colour, DMC, SSC, TA, TAC) of fruits of different ripeness. The models were first built by fruit variety, and then on the data obtained on the more mature and immature sides of the fruits, respectively, to explore its influence on the prediction accuracy.

PLSR modelling was also applied to predict the added extract concentration in fruit juices enriched to varying degrees. The models were first built using juice samples by fruit species, and then filtered according to simple, binary, and ternary blends when estimating CBE, GSE, PGE, and total extract content.

Identification of frequently occurring absorption bands in chemometric modelling

For each of the different chemometric approaches, the extent to which each wavelength supports the performance of the current modelling approach was determined. The wavelengths relevant to each applied modelling method were identified based on the spectral peaks in the PCA loadings, SIMCA and LDA discriminating powers and PLSR regression vectors. By fruit species and analytical method, the frequency with which each variable (i.e., wavelength) occurs in the chemometric modelling was summarised together, using spectral windows of approximately 10 nm. The resulting absorbance bands with their corresponding incidence values were plotted on line diagrams.

4. RESULTS AND DISDUSSION

4.1. Determination of the stone fruit ripeness with NIR spectroscopy

This subsection presents a summary for each fruit type regarding the effectiveness of a hand-held NIR spectrometer in predicting the ripeness and certain quality attributes of stone fruits. In all cases, PCA-LDA and PLSR models were developed by variety based on whole dataset collected on both mature and immature, as well as on the data respectively by measuring sides after optimised spectral pre-treatment.

4.1.1. Determination of sweet cherry ripeness

For the sweet cherries harvested at various stages of ripeness, a total of 26 and 21 pre-classified sample sets were analysed, respectively. The colours of the sweet cherries ranged from a completely immature green to close to overripe deep red. The pre-classified fruit samples were graded into six ripeness clusters. PCA-LDA classification models have shown in both varieties that the different ripeness levels were distinctly separated. The average correct classification rates during model validation were between 42.5 - 55.5% for the BB variety, and between 48.8 - 78.0% for the VC variety.

The most accurate PLSR models were obtained during the prediction of average L*, dry matter, soluble solid, and anthocyanin content, for both sweet cherry varieties. For the BB variety, DMC prediction accuracy was with a maximal R^2 of 0.88 - 0.83 and RMSE of 2.07 - 2.50% m/m. The prediction of SSC was achieved with an R^2 of 0.89 - 0.86 and RMSE of 1.09 - 1.23% brix. The prediction of TAC was achieved with an R^2 of 0.86 - 0.83 and RMSE of 12.14 - 13.51 mg/ L during calibration and validation, respectively. For the VC variety, the SSC prediction accuracy was a maximal R^2 of 0.95 - 0.93 and RMSE of 0.69 - 0.79% brix. The prediction of TAC was achieved with an R^2 of 0.91 - 0.87 and RMSE of 16.20 - 19.86 mg/ L. The prediction of average L* was achieved with an R^2 of 0.83 - 0.78 and RMSE of 4.76 - 5.44 during calibration and validation, respectively.

4.1.2. Determination of sour cherry ripeness

For the sour cherries harvested at various stages of ripeness, a total of 20 and 21 pre-classified sample sets were analysed, respectively. The colours of the cherries ranged from a light pink to a ripe deep red. The pre-classified fruit samples were graded into four ripeness clusters. PCA-LDA classification models have shown that the different ripeness levels distinctly separated. The average correct classifications during model validation were between 76.8 - 82.4% for the KJ variety, and between 78.30 - 80.9% for the UF variety. Misclassification typically occurred at adjacent ripeness levels.

The most accurate PLSR models were obtained during the prediction of average L*, b*, dry matter or soluble solid, and anthocyanin content, for both sour cherry varieties. For the KJ variety, DMC prediction accuracy was a maximal R^2 of 0.79 - 0.72 and RMSE of 1.47 - 1.67% m/m. The prediction of TAC was achieved with an R^2 of 0.91 - 0.87 and RMSE of 15.14 - 18.03 mg/ L. The prediction of average b* was achieved with an R^2 of 0.93 - 0.91 and RMSE of 1.26 - 1.51 during calibration and validation, respectively. For the UF variety. The prediction of SSC was achieved with a maximal R^2 of 0.87 - 0.83 and RMSE of 0.98 - 1.10% brix. The prediction of TAC was achieved with an R^2 of 0.89 - 0.87 and RMSE of 18.67 - 20.98 mg/ L. The prediction of average b* was achieved with an R^2 of 0.91 - 0.89 and RMSE of 1.54 - 1.78 during calibration and validation.

4.1.3. Determination of plum ripeness

For the plums harvested at different stages of ripeness, a total of 20 and 20 pre-classified sample sets were analysed. The colour of the plums ranged from unripe green to a dark purple. The pre-classified fruit samples were put into five and four ripeness clusters, respectively by variety. The sample populations were characterized by a significant presence of unripe fruits. PCA-LDA models also showed distinct separation. The average correct classifications during model validation were between 60.1 - 70.7% for the EL variety, and between 58.7 - 68.0% for the ST variety. Misclassification typically occurred at adjacent ripeness levels.

The most accurate PLSR models were obtained during the prediction of colour properties, soluble solids an acidity, for both plum varieties. For the EL variety, the prediction of SSC was achieved with a maximal R^2 of 0.97 - 0.95 and RMSE of 0.32 - 0.41% brix The prediction of TA was achieved with an R^2 of 0.97 - 0.95 and RMSE of 0.58 - 0.74 mg/ g. The prediction of average b* was achieved with an R^2 of 0.92 - 0.88 and RMSE of 2.40 - 2.84 during calibration and validation, respectively. For the ST variety. The prediction of SSC was achieved with a maximal R^2 of 0.93 - 0.86 and RMSE of 0.40 - 0.55% brix The prediction of TA was achieved with an R^2 of 0.94 - 0.90 and RMSE of 0.52 - 0.66 mg/ g. The prediction of average L* was achieved with an R^2 of 0.86 - 0.82 and RMSE of 2.75 - 3.12 during calibration and validation, respectively.

4.2. Detection of Monilinia contamination on stone fruits based on spectral characteristics

This section summarizes the effectiveness of using a hand-held NIR device and hyperspectral imaging to distinguish between differently infected and stored sour cherries and plums. Classification models were developed by variety and treatment group, after optimised spectral pre-treatment.

4.2.1. Detection results with a hand-held NIR spectrometer

Investigation results on sour cherries

During the preliminary investigation of the spectral data, only the UF sour cherries scanned with a hand-held spectrometer revealed observable separation trends according to sample treatment. The impact of storage temperature was evident, whereas the method of sample preparation is less, and no significant difference was found.

As a supervised classification, optimised PCA-LDA modelling was used to determine the accuracy with which the initial *Monilinia* conidium concentration can be determined based on the NIR spectra of sour cherries. For this classification, spectra recorded the day after inoculation were utilized, further filtered based on sample preparation and storage conditions. For the samples stored under refrigerated conditions, the average correct classification rates were between 63.1 - 85.1% during calibration and between 30.5 - 42.0% during validation. For the samples stored at room temperature, the average correct classification rates during calibration were between 58.2 - 75.4%, while during validation, they ranged from 23.5 to 31.5%.

Due to the reproductive characteristics of the *M. fructigena* involved in the experiments, only the samples infected through injury and stored at room temperature exhibited fungal activity. Considerable variability was observed for fruit showing signs of rotting even among the parallelly prepared samples. This may be attributed to the structural and compositional inhomogeneity of the cherries. It was considered important to examine the spectral trend of samples showing signs of brown rot on different days of storage. For this, optimised PCA-LDA modelling was employed using only the spectra of those samples that exhibited signs of decay during the 7-day long storage. Prior modelling, we sample specifically filtered the data corresponding to the day of appearance of the rot

(marked as “day 0”) ± 2-day interval and used this information to develop the classification models. The average correct classification rates during model building and validation for the EB variety were 77.2 and 48.2%, and the UF variety were 49.1 and 31.7%, respectively. A higher degree of misclassification predominantly occurred between the data of adjacent days.

Investigation results on plums

During the preliminary investigation of the spectral data, only the TP plums scanned with a hand-held spectrometer revealed observable separation trends according to sample treatment. The impact of storage temperature was evident, whereas the method of sample preparation is less, but no significant difference was found.

Optimised PCA-LDA modelling was used to determine the accuracy with which the initial *Monilinia* conidium concentration can be determined based on the NIR spectra of plums. For this classification, spectra recorded the day after inoculation were utilized, further filtered based on sample preparation and storage conditions. For the samples stored under refrigerated conditions, the average correct classification rates were between 74.2 - 92.9% during calibration and between 34.0 - 50.4% during validation. For the samples stored at room temperature, the average correct classification rates during calibration were between 77.8 - 89.4%, while during validation, they ranged from 39.2 to 51.6%.

Due to the reproductive characteristics of the *M. fructigena* involved in the experiments, only the samples infected through injury and stored at room temperature exhibited fungal activity. Unlike the sour cherries, several plum samples showed signs of rotting even with relatively low initial conidium contamination. This difference can be partly attributed to the lower acidity of plums compared to sour cherries, and thus have more favourable conditions for fungal growth. The acidity in fruits often acts as a natural inhibitor to fungal proliferation. It was considered important to examine the spectral trend of samples showing signs of brown rot on different days of storage. For this, optimised PCA-LDA modelling was employed using only the spectra of those samples that exhibited signs of decay during the 7-day long storage. Prior modelling, we sample specifically filtered the data corresponding to the day of appearance of the rot (marked as “day 0”) ± 2-day interval and used this information to develop the classification models. The average correct classification rates during model building and validation for the TD were 61.0 and 49.6% and TP variety were 38.9 and 25.3%, respectively. The overlap of the calculated data points, especially for the TP variety, is also reflected in the classification results. A high degree of misclassification mostly occurred between adjacent days for the TD variety.

4.2.2. Detection results with a hyperspectral imaging

Investigation results on sour cherries

The preliminary investigation of sour cherry data acquired with the hyperspectral imaging system revealed overlapping, at the same time consistent trends according to sample treatment, however no significant difference was found among sample groups.

Optimised PCA-LDA modelling was applied after filtering the data as detailed previously to determine the accuracy with which the initial *Monilinia* conidium concentration can be distinguished based on the hyperspectral data of sour cherries. For the samples stored under refrigerated conditions, the average correct classification rates were between 85.5 - 98.0% during calibration and between 33.1 - 53.3% during validation. For the samples stored at room temperature, the average correct classification rates during calibration were between 61.6 - 89.1%, during validation, they ranged from 32.5 - 46.3%. The classification accuracy of the HSI was better than that of the data recorded with the hand-held NIR instrument.

For these samples, we also observed that they showed visible signs of *Monilinia* infection with quite high variability. Regardless, the data filtering before PCA-LDA classification was performed as detailed previously, only on the hyperspectral data of sour cherries. The classification accuracies during model building and validation for the EB variety were 72.6 and 25.9% and for the UF variety were 86.5, 57.4%, respectively.

Investigation results on plums

The preliminary investigation of plum data acquired with the hyperspectral imaging system revealed overlapping, at the same time consistent trends according to sample treatment, however no significant difference was found among sample groups.

Optimised PCA-LDA modelling was applied after filtering the data as detailed previously to determine the accuracy with which the initial *Monilinia* conidium concentration can be distinguished based on the hyperspectral data of plums. For the samples stored under refrigerated conditions, the average correct classification rates were between 83.8 - 96.9% during calibration and between 50.0 - 69.5% during validation. For the samples stored at room temperature, the average correct classification rates during calibration were between 81.6 - 100%, while during validation, they ranged from 42.8 to 75.2%. It was also true for plums that the classification accuracy of the HSI was better than that of the data recorded with the hand-held NIR instrument.

For these samples, we also observed that they showed visible signs of *Monilinia* infection with quite high variability. Regardless, the same methods of data filtering prior PCA-LDA classification was performed as detailed previously, only on the hyperspectral data of sour cherries. The classification accuracies during model building and validation for the TD variety were 65.3 and 46.5% and TP variety were 57.3 and 33.5%, respectively.

4.3. Determination of fruit juice enrichment with NIR spectroscopy

This section summarises the accuracy achieved using hand-held and benchtop NIR spectroscopic devices in the detection and prediction of plant extracts added to fruit juices in various combinations and concentrations. The modelling results based on spectra recorded with the different instruments are presented pairwise, as the same samples were analysed with both instuments.

4.3.1. Detection results on sour cherry juices

The preliminary investigation of the spectral data of sour cherry juices obtained with the two different spectroscopic instruments highlighted fundamentally different measurement setups. Optimised PCA-LDA models revealed a logical overlap among the sour cherry juice blends. Particularly in the modelling based on data of the benchtop NIR instrument, it was evident that as the complexity of the blends increases, the data points representing these samples show a greater degree of superimposition of points corresponding to samples of partially similar compositions. The average correct classification for the hand-held device data were 66.65 and 42.45%, while for the benchtop spectrometer data 71.67 and 56.33% during model building and validation, respectively.

It was considered important to investigate the accuracy with which all the added extract content could be distinguished in sour cherry juices, regardless of the composition of blends involved. Also optimised PCA-LDA modelling was also applied for this purpose. The results showed evident separation trend according to total extract content. The classification accuracies for the hand-held device data were 71.73 and 49.62%, while for the benchtop spectrometer data 76.02 and 58.29% during model building and validation, respectively. Misclassification was typically to adjacent lower or higher concentration levels.

PLSR modelling was conducted to predict the concentrations of various extracts added to sour cherry juices. In the case of the hand-held NIR device, the prediction of CBE was made with Rcv^2 between 0.46 – 0.93; RMSEcv between 0.07 – 0.42 g/ 100 mL. The prediction of GSE was made with Rcv^2 between 0.65 – 0.93; RMSEcv between 0.07 – 0.34 g/ 100 g/mL. The prediction of PGE was made with Rcv^2 between 0.66 – 0.93; RMSEcv between 0.07 – 0.31 g/ 100 mL. The prediction of total extract content was made with Rcv^2 between 0.66 – 0.93; RMSEcv between 0.22 – 0.49 g/ 100 mL. In the case of the benchtop NIR instrument, the prediction of CBE was made with Rcv^2 between 0.86 – 0.98; RMSEcv between 0.04 – 0.21 g/ 100 mL. The prediction of GSE was made with Rcv^2 between 0.90 – 0.98; RMSEcv between 0.04 – 0.23 g/ 100 g/mL. The prediction of PGE was made with Rcv^2 between 0.86 – 0.98; RMSEcv between 0.04 – 0.16 g/ 100 mL. The prediction of total extract content was made with Rcv^2 between 0.86 – 0.98; RMSEcv between 0.12 – 0.32 g/ 100 mL.

4.3.2. Detection results on plum juices

The preliminary investigation of the spectral data of plum juices obtained with the two different spectroscopic instruments highlighted fundamentally different measurement setups. Optimised PCA-LDA models revealed less logical overlap among the juice blends in comparison to sour cherry juices. In the modelling based on data of the benchtop NIR instrument, it is more pronounced that as the complexity of the blends increases, the data points representing these samples show a greater degree of superimposition of points corresponding to samples of partially similar compositions. The classification accuracies for the hand-held device data were 53.11 and 27.04%, while for the benchtop spectrometer data 55.08 and 34.04% during model building and validation, respectively.

It was considered important to investigate the accuracy with which the extract content of all the added extracts could be distinguished in plum juices, regardless of the composition of blends involved. The optimised PCA-LDA results showed evident separation trend according to total extract content. The classification accuracies for the hand-held device data were 58.07 and 41.45%, while for the benchtop spectrometer data 66.96 and 46.29% during model building and validation, respectively. Misclassification was typically to adjacent lower or higher concentration levels. Misclassification was more pronounced to adjacent lower or higher concentration levels.

PLSR modelling was conducted to predict the concentrations of various extracts added to plum juices. In the case of the hand-held NIR device, the prediction of CBE was made with Rcv^2 between 0.53 – 0.84; RMSEcv between 0.13 – 0.59 g/ 100 mL. The prediction of GSE was made with Rcv^2 between 0.42 – 0.93; RMSEcv between 0.12 – 0.43 g/ 100 g/mL. The prediction of PGE was made with Rcv^2 between 0.47 – 0.93; RMSEcv between 0.12 – 0.62 g/ 100 mL. The prediction of total extract content was made with Rcv^2 between 0.77 – 0.93; RMSEcv between 0.23 – 0.40 g/ 100 mL. In the case of the benchtop NIR instrument, the prediction of CBE was made with Rcv^2 between 0.61 – 0.98; RMSEcv between 0.04 – 0.54 g/ 100 mL. The prediction of GSE was made with Rcv^2 between 0.59 – 0.98; RMSEcv between 0.04 – 0.37 g/ 100 g/mL. The prediction of PGE was made with Rcv^2 between 0.71 – 0.98; RMSEcv between 0.04 – 0.32 g/ 100 mL. The prediction of total extract content was made with Rcv^2 between 0.87 – 0.98; RMSEcv between 0.13 – 0.28 g/ 100 mL.

5. CONCLUSIONS AND RECOMMENDATIONS

As part of the doctoral research, multivariate statistical modelling was conducted based on NIR spectroscopy and hyperspectral imaging with the aim of mapping the key to effective application of the technique in the case of stone fruits and their products.

Sweet cherries, sour cherries and plums intended for the non-destructive assessment of fruits' physiological ripeness were harvested in a very inhomogeneous state according to ripeness. This allowed a wide spectrum of maturation to be studied, but also resulted in high variability in the outcomes. The former statement was mainly true for cherries. Sorting fruit by visually perceived ripeness (pre-classified samples) was often a challenge during the preparation of the measurements, as the available fruit stock did not have an equal quantitative distribution of fruit at different ripeness levels, therefore the number of fruits tested was also unequal in the larger ripeness clusters. To record the spectral characteristics of the fruits, a hand-held NIR device was employed, which is an easy-to-use tool for field studies in fruit production practice. The spectra were recorded on the more mature and immature sides of the fruits to test the effect of scanning location on the accuracy of prediction models.

For qualitative modelling, i.e., classification according to ripeness, spectral pre-treatment and NrPC-optimised LDA was used. To predict some of the fruits' value-measuring properties, spectral pre-treatment and NrLV-optimised PLS regression was employed. The results show that the accuracy of the prediction models was influenced by whether they are based on complete or partial datasets. However, for the latter case, if they were built on the data of more mature or the immature side can be dependent or sensitive to the variety of fruit and component under estimation.

Different varieties of sour cherries and plums were included in the experiments to detect Monilinia causing brown rot on fruits' surface. The spectra were obtained at three measurement points with the hand-held NIR device, and from nine surface areas with hyperspectral image processing. The latter's line scanning (push-broom) operation allowed fruit to be inspected without contact, as if they were moving on a conveyor belt. Separate sample sets were prepared for the two different instrumental analyses because the illuminating light of the HSI system used was very intense. Of the fruit infected with *M. fructigena* conidia in different ways and to different extents, only wound-infected samples stored above 20 °C showed signs of rotting and conidia formation. Within these sample sets, contrary to expectations, the "response" to infection of fruit that had been similarly inoculated and stored was different. Some fruits showed "meaningful" signs earlier, others later, if at all. In the recorded spectra, the increased amount of conidia on the surface of the fruit and the "dripping" of the wounds was disturbing. To reduce the effect of noise, various spectral pre-treatment techniques have been employed prior to qualitative modelling, otherwise the differentiation would have been based on light scattering only.

In general, there was a considerable, but not significant, divergence in the SIMCA models when the different modes of inoculation and storage were evaluated together on certain days of storage. The PCA-LDA models for classification according to initial conidia concentration based on spectra recorded on the first day of storage, showed varying classification accuracy, but it was generally true that HSI gave more accurate models. Interestingly, for samples of EB, UF and TP varieties, the classification of the same sample sets by instrument was the most accurate. The highest average correct classification according to the appearance time of visible signs of *Monilinia* infection was based on hand-held NIR spectra for EB and TD samples, and HSI spectra for UF and TP samples.

The examination of fruit juices enriched with various extracts was conducted in transflexion and transmission arrangements using a hand-held NIR and a benchtop FT NIR spectrometer. The same samples were examined with both instruments. The characteristic of the prepared fruit juices was that the dosed extract did not always completely dissolve and the powdery particles tended to settle in the sample containers. After homogenization, when loading the sample solutions, we aimed to analyse a solution free of interfering components. During the qualitative and quantitative analysis of spectral data, spectral pre-processing, NrPC- or NrLV-optimised PCA-LDA and PLSR modelling were applied, respectively.

The results show that chemometric modelling based on data recorded by the benchtop instrument resulted in more accurate classification and extract concentration prediction. This can be attributed, on the one hand, to the instrument's resolution, and on the other hand, to the measurement setup, highlighting that the transmission measurement approach is better suited for measuring transparent liquids like the juices we had. Comparing the modelling results of the examined fruit juices, it was found that sour cherry juices exhibited better model fitting. This is suspected to be due to some unidentified sample preparation anomaly in the case of plum juice samples.

Chemometric modelling results based on spectra recorded with HSI and NIR instruments show relatively high variability, especially during classifications. The primary reason for this is the naturally high variability of the fruits, despite the sample replicates. Our research, based partly on the development of measurement techniques and partly on statistical methods, is of great importance as it is based on the investigation of economically important fruits, for which there is very limited source material available, both in literature and in practice, for the non-destructive examination. Small-scale handheld NIR instruments can be used for on-site inspections, while line scan recording of HSI can support continuous production processes. In addition, prominent absorption bands obtained from chemometric modelling can contribute to the development of target instruments.

Based on the above summary, we have the following suggestions for the extension of studies:

- Preparation of fruit studies for larger sample sets,
- Involvement of untested factors in the modelling (e.g., different origin, season, etc.),
- Pre-sorting of fruits not only on the basis of their visual characteristics,
- Very precise setting of fruit storage and measurement conditions,
- Implementation of wavelength selection methods prior chemometrics,
- Involvement of other chemometric methods in data analysis (e.g., PLS-DA, SVM, k-NN),
- Calibration transfer between precision benchtop and hand-held instruments,
- Model testing with completely independent sample sets.

6. NEW SCIENTIFIC FINDINGS

In the new scientific results, the handheld near infrared (NIR) spectrometer used in fruit ripeness studies refers to NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan). In the Monilinia detection studies, the hand-held NIR instrument refers to NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan), and the hyperspectral imaging system refers to Headwall XEVA-1648 XC134. In the fruit juice studies, hand-held NIR device refers to MicroNIR (Viavi, Scottsdale, USA), and benchtop spectrometer refers to NIRFlex N-500 (Büchi Labortechnik AG, Flawil, Switzerland). Classification modelling refers to principal component analysis-based linear discriminant analysis (PCA-LDA), and predictive modelling refers to partial least squares regression (PLSR).

New scientific findings on the determination of stone fruit ripeness with hand-held NIR spectrometer (950-1650 nm)

1. The efficiency with which a hand-held NIR spectrometer could classify stone fruits according to ripeness has been determined.
- For sweet cherries, the classification models performed with up to 91.7 and 78.0% accuracy during model building and validation, respectively.
- For sour cherries, the classification models performed with up to 87.8 and 82.4% accuracy during model building and validation, respectively,
- For plums, the classification models performed with up to 82.1 and 70.7% accuracy during model building and validation, respectively.

2. The efficiency with which a hand-held NIR spectrometer could predict dry matter content of stone fruits of different ripeness has been determined.
- For sweet cherries, the modelling and validation was performed with a maximal Rc^2 of 0.88 and RMSEc of 2.07% m/m, Rcv^2 of 0.83 and RMSEcv of 2.50% m/m, respectively.
- For sour cherries, the modelling and validation was performed with a maximal Rc^2 of 0.79 and RMSEc of 1.47% m/m, Rcv^2 of 0.72 and RMSEcv of 1.67% m/m, respectively.
- For plums, the modelling and validation was performed with a maximal Rc^2 of 0.45 and RMSEc of 1.02% m/m, Rcv^2 of 0.35 and RMSEcv of 1.11% m/m, respectively.

3. The efficiency with which a hand-held NIR spectrometer could predict soluble solid content of stone fruits of different ripeness has been determined.
- For sweet cherries, modelling and validation was performed with a maximal Rc^2 of 0.95 and RMSEc of 0.69% brix, Rcv^2 of 0.93 and RMSEcv of 0.79% brix, respectively.
- For sour cherries, modelling and validation was performed with a maximal Rc^2 of 0.87 and RMSEc of 0.98% brix, Rcv^2 of 0.83 and RMSEcv of 1.10% brix, respectively.
- For plums, modelling and validation was performed with a maximal Rc^2 of 0.97 and RMSEc of 0.32% brix, Rcv^2 of 0.95 and RMSEcv of 0.41% brix, respectively.

New scientific finding on the spectral detectability of Monilinia contamination in stone fruits with a hand-held NIR spectrometer or hyperspectral imaging (1000-1650 nm)

4. For the first time in the scientific literature, the performance of a hand-held NIR spectrometer for the detection of *Monilinia fructigena* on the surface stone fruits (with or without injury, stored at refrigerated or room temperature) has been determined based on spectral data recorded on the first

day of storage after inoculation. Classification models were developed separately by storage condition

- to discriminate sour cherries of four conidial contamination levels (in tenfold dilutions: ~ 100-10-1-0.1 conidium/μL), when classification accuracies for "Érdi bőtermő" sour cherries were between 63.1-77.6% and 23.5-34.1%, for "Újfehértói" cherries were between 58.2-85.1% and 24.6-42.0% during model building and validation, respectively.
- to discriminate plums of three conidial contamination levels (in tenfold dilutions: ~ 100-10-1 conidium/μL), when classification accuracies for "Topend" plums were between 74.2-84.2% and 34.0-50.4%, for "Topend plus" plums were between 78.9-92.9% and 35.1-51.6% during model building and validation, respectively.

5. For the first time in the scientific literature, the performance of a hyperspectral imaging for the detection of *Monilinia fructigena* on the surface stone fruits (with or without injury, stored at refrigerated or room temperature) has been determined based on spectral data recorded on the first day of storage after inoculation. Classification models were developed separately by storage condition

- to discriminate sour cherries of four conidial contamination levels (in tenfold dilutions: ~ 100-10-1-0.1 conidium/μL), when classification accuracies for "Érdi bőtermő" sour cherries were between 61.6-85.6% and 33.1-45.0%, for "Újfehértói" cherries were between 83.4-98.0% and 32.5-53.3% during model building and validation, respectively.
- to discriminate plums of three conidial contamination levels (in tenfold dilutions: ~ 100-10-1 conidium/μL), when classification accuracies for "Topend" plums were between 81.6-97.1% and 42.8-75.2%, for "Topend plus" plums were between 78.9-87.1-100% and 50.0-79.3% during model building and validation, respectively.

New scientific findings on the predictability of fruit juice enrichment with NIR spectroscopy (1000-1650 nm)

6. The performance of a hand-held NIR spectrometer for the prediction of fruit juice enrichment with plant extracts has been determined.

- In simple sour cherry juice blends, cranberry extract was predicted with an Rcv^2 of 0.92 and RMSEcv of 0.25 g/100 mL, grape seed extract content with an Rcv^2 of 0.90 and RMSEcv of 0.27 g/100 mL, and pomegranate extract with an Rcv^2 of 0.87 and RMSEcv of 0.31 g/100 mL.
- In simple plum juice blends, cranberry extract was predicted with an Rcv^2 of 0.53 and RMSEcv of 0.59 g/100 mL, grape seed extract content with an Rcv^2 of 0.76 and RMSEcv of 0.42 g/100 mL, and pomegranate extract with an Rcv^2 of 0.47 and RMSEcv of 0.62 g/100 mL.

7. The performance of a benchtop NIR spectrometer for the prediction of fruit juice enrichment with plant extracts has been determined.

- In simple sour cherry juice blends, cranberry extract was predicted with an Rcv^2 of 0.97 and RMSEcv of 0.13 g/100 mL, grape seed extract content with an Rcv^2 of 0.92 and RMSEcv of 0.23 g/100 mL, and pomegranate extract with an Rcv^2 of 0.97 and RMSEcv of 0.15 g/100 mL.
- In simple plum juice blends, cranberry extract was predicted with an Rcv^2 of 0.61 and RMSEcv of 0.54g/100 mL, grape seed extract content with an Rcv^2 of 0.90 and RMSEcv of 0.27 g/100 mL, and pomegranate extract with an Rcv^2 of 0.98 and RMSEcv of 0.18 g/100 mL.

7. LIST OF PUBLICATIONS IN THE FIELD OF STUDY

First author publications

Flóra Vitális, Juan Pablo Aguinag Bósquez, Mátyás Lukács, Marietta Petróczy, Marietta Fodor, Zoltán Gillay, Zoltán Kovács (2024). Development of state-of-the-art correlative rapid methods for the non-destructive control of fruit products. Scientia et Securitas, 4(4), 258-264. (DOI: 10.1556/112.2023.00202)

Flora Vitalis, Jelena Muncan, Sukritta Anantywittayanon, Zoltan Kovacs, Roumiana Tsenkova (2023). Aquaphotomics Monitoring of Lettuce Freshness During Cold Storage. *Foods*, 12(2): 258. (DOI: 10.3390/foods12020258) – **Q1** (2023)

Flora Vitalis, David Tjandra Nugraha, Balkis Aouadi, Juan Pablo Aguinaga Bósquez, Zsanett Bodor, John-Lewis Zinia Zaukuu, Tamás Kocsis, Viktoria Zsom-Muha, Zoltan Gillay, Zoltan Kovacs (2021). Detection of Monilia Contamination in Plum and Plum Juice with NIR Spectroscopy and Electronic Tongue, *Chemosensors*, 9(12), 355. (DOI: 10.3390/chemosensors9120355) – **Q2** (2023)

Vitális Flóra (2021). Növényi eredetű élelmiszerek eredetiségének meghatározása közeli infravörös spektroszkópiával. *Táplálkozástudományi Morzsák Hírlevél*, 4(3), pp. 11-13. (ISSN: 2630-8975)

Flora Vitalis, John-Lewis Zinia Zaukuu, Zsanett Bodor, Balkis Aouadi, Géza Hitka, Timea Kaszab, Viktoria Zsom-Muha, Zoltan Gillay, Zoltan Kovacs (2020). Detection and Quantification of Tomato Paste Adulteration Using Conventional and Rapid Analytical Methods, *Sensors*, 20(21), 6059. (DOI: 10.3390/s20216059) – **Q1** (2023)

Vitális Flóra, Bodor Zsanett, John-Lewis Zinia Zaukuu, Bázár György, Kovács Zoltán (2019)**.** Aquaphotomics: a közeli infravörös spektroszkópia innovatív, víz központú alkalmazása [Aquaphotomics: an innovative application of near-infrared spectroscopy focusing on water]. *Élelmiszervizsgálati Közlemények*, 65(4). pp. 2672-2687., 16 p. (ISSN: 2676-8704) – **Q4** (2023)

Co-authored publications

Gergo Szabo, **Flora Vitalis**, Zsuzsanna Horvath-Mezofi, Monika Gob, Aguinaga Juan Palblo Bosquez, Zoltan Gillay, Tamas Zsom, Lien Le Puong Nguyen, Geza Hitka, Zoltan Kovacs, Laszlo Friedrich (2023). Application of Near Infrared Spectroscopy to Monitor the Quality Change of Sour Cherry Stored Under Modified Atmosphere Conditions. *Sensors*. 23(1):479. (DOI: 10.3390/S23010479) – **Q1** (2023)

Lívia Darnay, **Flóra Vitális**, Anna Szepessy, Dávid Bencze, Tamás Csurka, József Surányi, Ferenc Firtha, Péter Laczay (2022). Comparison of different visual methods to follow the effect of milk heat treatment and microbial transglutaminase on appearance of semi-hard buffalo cheese. *Food Control*, 139, 109049. (DOI: 10.1016/j.foodcont.2022.109049) – **D1** (2023)

Balkis Aouadi, **Flora Vitalis**, Zsanett Bodor, John-Lewis Zinia Zaukuu, Istvan Kertesz, Zoltan Kovacs (2022). NIRS and Aquaphotomics Trace Robusta-to-Arabica Ratio in Liquid Coffee Blends, *Molecules*, 27(2), 388. (DOI: 10.3390/molecules27020388) – **Q1** (2023)

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