Development of state-of-the-art correlative rapid methods for the non-destructive control of fruit products

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Summary

Stone fruits play a significant role in fruit breeding, gastronomy, and industry, largely due to their beneficial nutritional composition. Ensuring the quality of these seasonal fruits poses a substantial challenge due to intensive customer demand. In this study, through the example of sour cherries, we demonstrate how quality variations can be effectively controlled at specific critical points along the supply chain. Paired with various chemometric methods, near infrared spectroscopy reliably classified fruits based on their harvest maturity, different stages of *Monilinia* brown rot, and predicted the content of added foreign fruit extracts for functionality enhancement in sour cherry juices. The applied approach supports agricultural digitisation and food safety.

Keywords: fruit ripeness, brown rot, authentication, near infrared spectroscopy, chemometrics

Korrelatív gyors módszerek fejlesztése gyümölcstermékek roncsolásmentes vizsgálatára

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Összefoglalás

A meggy a csonthéjas gyümölcsök nemesítésének és termelésének egyik legfontosabb alanya. A megnövekedett fogyasztói kereslet a kedvező beltartalmi összetételének tudható be, “szuperélelmiszerként” tartják számon. A viszonylag alacsony cukor-sav aránya miatt a meggyet főként feldolgozott formában fogyasztjuk. A hatékony feldolgozást nagy mértékben befolyásolja a nyersanyag érettségi és fiziológiai állapota. Az előbbi a termék kihozatalra, összetételre és funkcionalitásra, az utóbbi a stabilitásra van kihatással. Az ellátási láncban jelentkező veszteségek jelentős része romlási folyamatok miatt jelentkezik, amelyek közül az egyik legfontosabb a barna rothadást okozó *Monilinia* spp. Egy másik fontos szempont az élelmiszer eredetiségének biztosítása. A feldolgozásnak kimutatható hatása van a meggyből készült termékek funkcionalitására, aminek jogtalan manipulálása hamisítást feltételez. Az érési, romlási folyamatok és esetleges csalás korrelatív módszerekkel hatékonyan lekövethető. Kutatásunkban meggyek példáján keresztül mutatjuk be, hogy a közeli infravörös (NIR) spektroszkópia milyen eredményességgel alkalmazható gyümölcs érettség, *Monilinia fructigena* okozta barna rothadás és idegen növényi extraktumok roncsolásmentes kimutatásához gyümölcslevekben. A spektrumok gyűjtése kézi NIR műszerekkel történt a 900–1700 nm hullámhossztartományban gyümölcsök esetében diffúz reflexiós, míg gyümölcslevek esetében transflexiós elrendezésben. Az adatok elemzése a 950–1650 nm tartományban valósult meg spektrum előkezelést követően (pl. simítás, detrending, szóródási korrekciók, deriváltak). Főkomponens elemzést (PCA) alkalmaztunk előzetes feltérképezésként; PCA alapú lineáris diszkriminancia elemzést (LDA) végeztünk betakarítási érettség, korai *Monilinia* vagy idegen extrakttartalom kimutatásához; részleges legkisebb négyzetek regressziót (PLSR) a gyümölcsök egyes beltartalmi jellemzőinek, illetve idegen növényi extrakttartalom becsléséhez. Az érettségvizsgálati eredmények alapján a NIR spektrumokon alapuló PCA-LDA modellek lehetővé teszik a meggyek nagy pontosságú osztályozását betakarítási érettségük és lehetséges felhasználásuk szerint. A beltartalmi jellemzők becslésénél az összes oldott szárazanyag és az összes antocianin tartalom esetében adott pontos eredményt a PLSR. A *Monilinia* okozta barna rothadás azoknál a meggyeknél volt korai stádiumban kimutatható, amelyek vágott sebeit nagyságrendileg 100 és/vagy 10 konídium/µL koncentrációjú szuszpenzióval fertőztük és szobahőmérsékleten tároltuk. A NIR technikával eredményesen azonosítottuk a különböző idegen növényi extraktumokat és becsültük azok koncentrációját természetesen nagy színanyagtartalmú meggylevekben. Az alkalmazott roncsolásmentes, többszörösen felhasználható megközelítés támogatja a mezőgazdasági digitalizációt és hozzájárul a biztonságos és fogyasztók által elvárt minőségű élelmiszerek előállításához.

Kulcsszavak: gyümölcs érettség, barna rothadás, eredetvizsgálat, közeli infravörös spektroszkópia, kemometria

Introduction

Cherries are among the most popular varieties in the production and processing of stone fruits. The awareness of the health benefits associated with cherries has led to a notable rise in both their production and consumption. Among seasonal fruit crops, sweet cherries stand out, being one of the few remaining that generate significant seasonal in-store activity, unparalleled by other items in many markets *(Kappel et al. 2012)*. Cherries originated from European regions with a temperate climate, and the main producing countries of the recent years are Turkey, European Union and China *(FAO 2022; Shahbandeh 2023)*.

To meet customer needs and enhance production, various breeding programmes are underway; half of these focus on incorporating self-fertilisation while the other half aim to elevate fertility levels. This aimed at fortifying resistance against unfavourable environmental conditions, including susceptibility to issues like cracking and diseases in the post-bloom and preharvest phases. Furthermore, efforts were made to extend the harvesting season by up to three months through the introduction of novel early- and late-ripening cultivars *(Dondini–Lugli–Sansavini 2018)*. This paper focuses on sour cherry whose internationally renowned cultivation traditions are deeply rooted in Hungary *(Schuster et al. 2017)*.

Sour cherry, also known as tart cherry, is considered "superfood" due to its advantageous nutritional composition *(Alba C–Daya–Franck 2019)* that lies in its relatively low simple sugar and very high antioxidant content. Numerous investigations were conducted to profile the phytochemical composition of cherry cultivars *(Hegedűs et al. 2018; Khoo et al. 2011; Nemes et al. 2018; Papp et al. 2010)* also responsible for neuroprotective, anti-inflammatory, anti-cancer, anti-diabetes activities, and against cardiovascular diseases *(Blando–Oomah 2019)*.

Due to the relatively low soluble sugar-to-acid ratio (SSC/TA), the fruits are mainly consumed in processed form *(Ivanova et al. 2018)*. The efficient and preferably loss-free processing of fruit for various uses, whether for fresh consumption or preservation, would not be complete without knowing the ripeness and physiological state of the raw material. While the former influences production yield, composition and functionality of the product *(Cásedas et al. 2016)*, the latter affects product stability and safety. A significant proportion of losses in the supply chain, up to 50%, may be attributed to decay processes *(Elik et al. 2019)*. One of the most important threats to stone fruit and thus sour cherry production are blossom and twig wilt coupled with brown rot caused by *Monilinia* spp. *(Di Francesco–Mari 2018)*. *M. fructigena, M. laxa and M. fructicola* are considered economically the most significant species *(Hrustić et al. 2012)*, whose morphological and molecular characterisation *(Petróczy 2009; Petróczy–Szigethy–Palkovics 2012)*, as well as description of sporulation dynamics have been done by Hungarian researchers *(Holb–Szőke–Abonyi 2013)*.

Another important aspect in food quality and safety is the assurance of authenticity, that consumers have the quality and origin they expect on their tables. Industrial processing has been shown to have a significant impact on the composition of sour cherry products, in particular on phenolic compounds *(Toydemir et al. 2013)*. The masking of this and the marketing of a seemingly good quality product is motivated by illegal economic gain. Any incriminating product manipulation falls under the term of food fraud and food adulteration, as extensively detailed by *Spink et al. (2011, 2019), Moore–Spink–Lipp (2012) and Everstine–Spink–Kennedy (2013)*.

In practice, quality control lies on empirical methods and/or the determination of a set of quality indicators (e. g., fruit detachment, colour, firmness, microbial count, SSC/TA, etc.) *(Kállay et al. 2007)*, however, a significant amount of recent publications support the idea that non-destructive fingerprinting methods can be used to universally describe quality differences. One of the most important of these is the near infrared (NIR) spectroscopy, employed in agricultural research and industrial applications for almost hundred years *(Norris 1992)*. Regarding stone fruits and cherries, the NIR technique combined with multivariate statistics has been successfully applied to determine intrinsic and sensory attributes related to ripeness *(Escribano et al. 2017; Scalisi–O'Connell 2021; Shah et al. 2020)*, detect physical *(Shao et al. 2019)*, environmental *(Szabo et al. 2023)* and microbial damage during storage *(Vitalis, Bósquez et al. 2021; Vitalis, Tjandra Nugraha et al. 2021)*. Furthermore, the method provides accurate results in authenticating, and detecting possible adulteration of fruit juices *(Arendse et al. 2021; Wang et al. 2017)*.

Our aim is to develop innovative rapid measurement methods that can be applied to effectively determine the origin, physiological state and certain quality characteristics of fruits, and to detect and predict the extent of adulteration in fruit products. This article demonstrates the applicability of non-destructive NIR technique in determining fruit quality and product authenticity through the example of sour cherries.

Materials and methods

Samples and their preparation

Samples for the fruit ripeness study

Sour cherry samples were obtained from the Szatmár region (Hungary) for non-destructive determination of ripeness and thus the optimal harvest time of fruits. Sour cherries of the *Újfehértói* variety harvested at different stages of ripeness were included in the assessments. The fruits were sorted into 21 groups according to their visible colour indicating ripeness, and then classified into four large clusters according to the possible usage as listed below. Five fruits were analysed in each of the 21 maturity groups, resulting in 105 cherries in total to be analysed.

- (●) L1 – unripe/ initial colouration
- (●) L2 – intensive ripening – production of preserves
- (●) L3 – ripe – production of fresh/ juice/ concentrate/ jam / frozen products
- (●) L4 – fully ripe – production of fresh/ juice/ concentrate/ jam / frozen products

Samples for the brown rot detection study

In the experiment aimed to detect brown rot caused by *Monilinia spp*., also sour cherries of the *Újfehértói* variety were used. The investigation was performed using *M. fructigena* isolated from sour cherries. The fungal conidium propagation was also done on sour cherries. All these processes of isolation, propagation, conidium collection and suspension preparation were done in accordance with the work by *Petróczy (2009)*. The actual fruit sample preparation started with stem removal and sour cherries disinfection with ethanolic solution. Approximately 3 mm cuts were applied on the surface of some of the fruits with a sterile knife, and 20 µL fungal conidium suspension of the order of 100 conidia/µL, 10 conidia/µL, 1 conidia/µL or 0.1 conidia/µL was pipetted into each cut. These formed the sample group labelled as "Injury". Subsequently, 20 µL of the suspensions were instilled without injury into the next part of the fruits, these formed the "Intact" samples. The remaining cherries were not infected, and gave the "Control" samples. One half of the prepared fruits were stored for seven days at refrigerated (~5 °C) and the other half at room temperature (~24 °C) in controlled atmosphere. Five replicate fruits per sample group were prepared resulting a total of 90 [((1 control + 4 injury + 4 intact) × 2 storage conditions) × 5 repl.] samples to be analysed.

Samples for the fruit juice authentication study

In the investigation aimed to non-invasively assess the detectability of "foreign" extract dosage in fruits juices with an inherently high colour content, cranberry, grapeseed and pomegranate extracts were added in 0 to 2.5 g/ 100 mL to sour cherry juice (~22% SSC) made from concentrate (~64% SSC). Five and three replicate sample preparations were done for the pure (stock) and enriched juices, respectively, resulting a total of 59 [(3 extracts × 6 conc. levels × 3 repl.) + 5 stock juices] samples. All the samples were pipetted into centrifuge tubes of 15 mL, pasteurised in drying chamber (85 °C, 60 s), and refrigerated until analysis.

Applied methods

Reference measurements

Some of the intrinsic quality attributes of sour cherries were determined with destructive methods. The dry matter, the soluble solid, the titratable acid and the anthocyanin content determinations were performed in accordance with *Fodor (2022)*.

Non-destructive measurements

Near infrared (NIR) spectroscopy was used for the non-destructive analysis of sour cherries. The applied hand-held NIR reflectance spectrometer (NIR-S-G1, InnoSpectra Co., Hsinchu, Taiwan) allows even on-site spectrum acquisition. In the case of the ripeness study, the collection of the spectra was done on the ripe and unripe sides of the fruits, while in the *Monilinia* investigation, it was at three measurement points on the horizontal axis of the cherries. After each measurement, the contact surface of the instrument was disinfected with a wipe soaked in ethyl alcohol to avoid cross-contamination. The spectra of the fruits were recorded on each day of storage for seven days.

The fruit juice samples were analysed based on their transflectance spectra recorded with a hand-held NIR device (MicroNIR, Viavi, Scottsdale, USA). Regardless of the experiment, three consecutive spectra per measurement points were recorded in the wavelength range of 900–1700 nm.

Multivariate data analysis
Multivariate data analyses (chemometrics) were applied to the NIR spectra in the wavelength range of 950–1650 nm. To reduce the noise in the spectra, Savitzky–Golay smoothing *(Savitzky–Golay 1964)* coupled with other spectral pretreatments were used (e. g., scatter correction, detrending, derivatives) to optimise subsequent statistical modelling.

Principal Component Analysis (PCA) was used to compress significantly correlated NIR data into uncorrelated new variables (principal components, PCs) for preliminary pattern mapping, as well as for input calculation to subsequent classification. PCA-based linear discriminant analysis (PCA-LDA) was used as a supervised method to detect differences among samples according to grouping variables such as ripeness, signs of *Monilinia* in cherries, or the type and concentration of extract in sour cherry juices. Partial least squares regression (PLSR) was applied to predict fruit quality attributes or extract content in fruit juices based on the NIR spectra. All the predictive models were validated by the cyclic omission of fruits prepared in replicate in each sample group. This was repeated until all data had been involved in the modelling and validation at least once. The paper reports the best modelling results when the prediction error of the cross-validation was the lowest.

Results and discussion

Prediction of fruit ripeness

The non-destructive assessment of fruits allows to determine their ripeness even in the orchard without picking, or the most advantageous use of fruit already harvested according to ripeness. This indirectly helps to reduce agricultural losses from the outset. From the fruit received for testing, a total of four major maturity clusters could be formed based on their colour. Without specifically analysing the spectral data, it was observed that the light absorption of cherries increases in the wavelength range studied as the ripening progresses.

Figure 1a shows the PCA results and illustrates slight separation tendency according to fruit ripeness along the first two principal components that describe approximately 91.08% of the total variance. The LDA modelling support the correlation between ripening and change in absorption *(Figure 1b)*. The average correct classifications were 87.50 and 79.23% during model building and validation, respectively, when spectra recorded on both the ripened and unripe sides of the fruits were included in the modelling. Relatively higher misclassification happened to higher levels of ripeness.

NIR spectroscopy-based regression also enables the prediction of internal quality attributes without the need of damaging the fruits. *Table 1* summarises the predictability of some of the attributes investigated in sour cherries of different ripeness. Focusing on the coefficient of determination and root mean square error during model validation, the most accurate model fittings were found for soluble solid content and total anthocyanin content.

Determination of Monilinia contamination

The utilisation of portable NIR spectrometer in the study enables *in situ* measurements, providing a valuable tool for promptly identifying potentially hazardous fruit quantities due to microbiological risks. This greatly supports effective intervention, identification and removal of potential hotspots.

The spectrum recorded during the seven-day storage of sour cherries infected in various ways and stored under different circumstance is depicted in *Figure 2a*. The

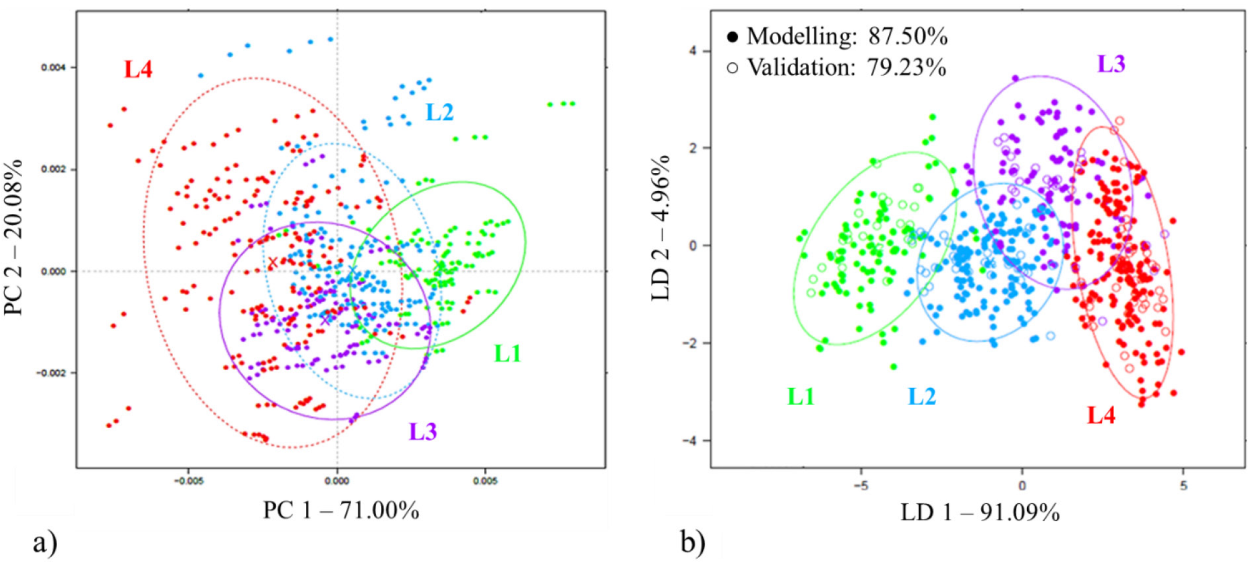

Figure 1 Unsupervised and supervised modelling of sour cherries: (a) PCA score plot; (b) PCA-LDA score plot when "fruit ripeness" was used as class variable.
Source: own edition

Table 1 | Prediction results of some of the sour cherry intrinsic quality attributes with PLSR

Quality attribute	Nr	NrLVs	Rc^2	RMSEc	Rcv^2	RMSEcv
Dry matter content (% *m/m*)	484	16	0.49	1.71	0.34	1.95
Soluble solid content (%)	492	16	0.86	1.00	0.82	1.12
Titratable acidity (mg/ g)	503	7	0.43	1.45	0.33	1.57
Total anthocyanin content (mg/ L)	515	16	0.86	21.49	0.83	23.73

Nr – number of observations; NrLVs – number of latent variables; Rc^2 and Rcv^2 – coefficient of determination during model calibration and validation; RMSEc and RMSEcv – root mean square error during model calibration and validation (g/ 100 mL)

raw spectra show significant overlap, but the clear impact of the fruit handling is evident. Generally, the undamaged and refrigerated fruits exhibited higher absorbance. Cherries infected through injury and stored at room temperature dried out more easily and quickly.

Monilinia fructigena, studied in the context of sour cherries, only induces fruit deterioration in the presence of surface damage, with its optimal multiplication temperature being above 20 °C *(Lichtemberg et al. 2014)*. Theory has also met practice in our samples, and showed signs of *Monilinia* conidium formation. This could be traced in the spectra, as the increasing number of conidia led to a greater level of scatter in the spectral data.

According to our observations, it was also influential to what extent the fruit was infected. Signs of proliferation could only be identified on those fruits where a suspension with a concentration of approximately 100 or 10 conidia/ µL was applied. It was believed that the early detectability of *Monilinia* is worth investigating in sour cherries genuinely threatened by brown rot.

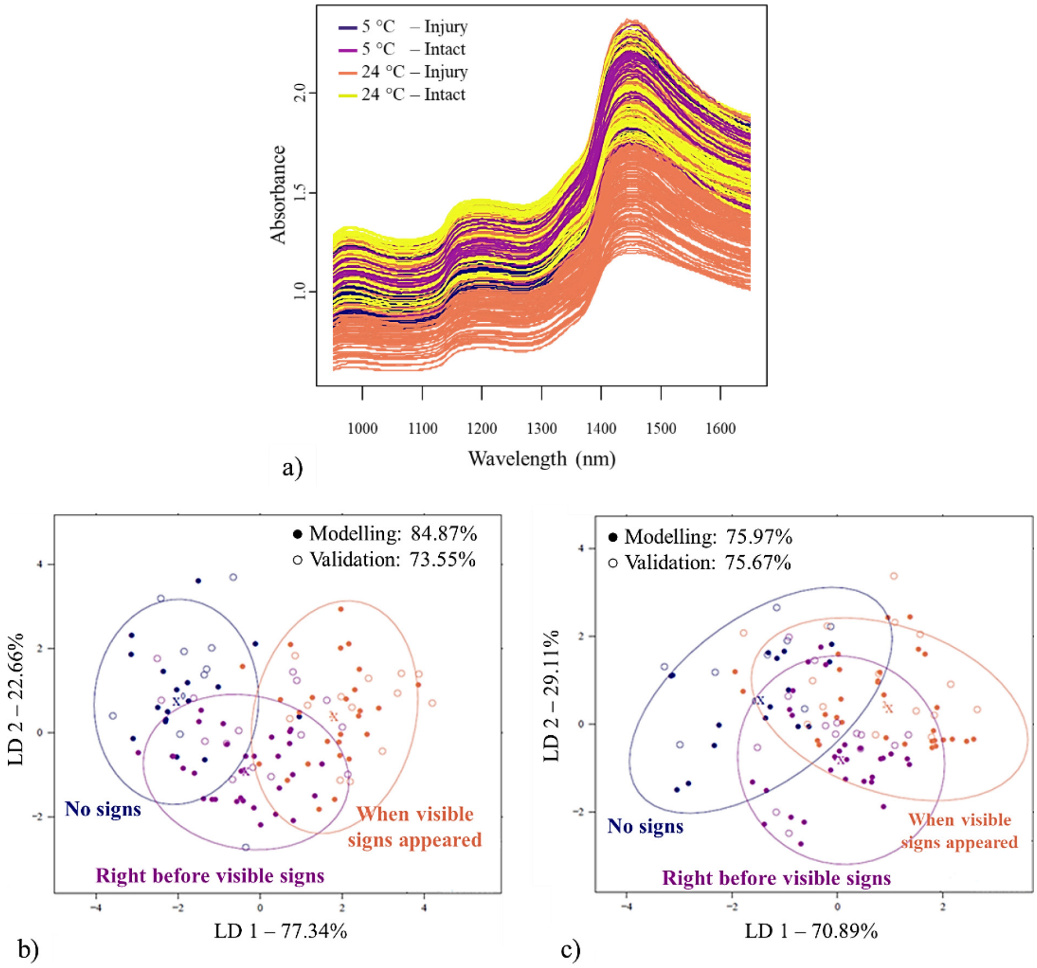

Figure 2 | Spectra recorded during the storage of sour cherries infected with *Monilinia* conidial suspensions in different ways (a); PCA-LDA score plot of cherries stored at 24 °C and infected trough injury with suspension of 170 conidia/µL (b), or 17 conidia/µL (c) when "visible signs of infection" was used as the class variable.

Source: own edition

Table 2 | Prediction results of fruit extract dosage in sour cherry juices with PLSR

Type of fruit extract	Nr	NrLVs	Rc^2	RMSEc	Rcv^2	RMSEcv
Cranberry extract	99	6	0.936	0.212	0.880	0.290
Grapeseed extract	99	5	0.933	0.217	0.896	0.271
Pomegranate extract	99	6	0.958	0.173	0.875	0.297

Nr – number of observations; NrLVs – number of latent variables; Rc^2 and Rcv^2 – coefficient of determination during model calibration and validation; RMSEc and RMSEcv – root mean square error during model calibration and validation (g/ 100 mL)

Accordingly, the available data was specifically filtered for the endangered sample sets. *Figure 2b* and *2c* illustrates the PCA-LDA results when visible signs of infection were in question. The results confirm that a higher conidium concentration accelerates the spread of infection, and overall, the infection could be traced with higher accuracy.

Detection of food fraud

To preserve the nutritional value of seasonal fruits various preservation methods are necessary. The raw material intended for processing undergoes physical, chemical, and biological changes as a result of various technological steps. In the assessment of cherry products, unwanted changes in functionality and colour are mainly attributed to important phenolic compounds *(Toydemir et al. 2013)*. While resolving these changes may not be always feasible, in certain instances, they are either masked or mitigated. However, if this occurs in an unauthorised, unacceptable manner and extent, it may suggest food fraud and/or adulteration.

Our model research focused on food safety, and investigated the detectability of foreign plant extracts when dosed in small concentrations (0–2.5 g/100 mL) in naturally dark-colored sour cherry juices. Using PCA-LDA modeling, the type of adulteration could be identified with up to 86.57 and 79.82% accuracy during model calibration and validation, respectively. When the model fitting of foreign extract content was in focus, the results yielded reasonably good predictive outcomes as shown in *Table 2*.

Conclusions

The study outlines some segments of extensive experiments aimed at assessing fruit ripeness, detecting brown rot, and verifying the authenticity of fruit juices applying handheld NIR spectroscopy and chemometrics. By utilising sour cherries as a model, the effective application and versatility of non-destructive NIR technology were showcased in addressing some of the crucial aspects of food quality and safety. We believe that the applied approach substantially bolsters agricultural digitalisation, improves food production, and helps ensure the provision of safe and expected high-quality food both within and beyond our borders.

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