

## Research Article

# Comparison between Hyperspectral Imaging and Chemical Analysis of Polyphenol Oxidase Activity on Fresh-Cut Apple Slices

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The assessment of the quality of fresh-cut apple slices is important for processing, storage, market value, and consumption. Determination of polyphenol oxidase activity (PPO) in apples is critical for controlling the quality of the final product (i.e., dried apples and juices). Hyperspectral imaging (HSI) is a nondestructive, noncontact, and rapid food quality assessment technique. It has the potential to detect physical and chemical quality attributes of foods such as PPO of apple. The aim of this study was to investigate the suitability of HSI in the visible and near-infrared (VIS-NIR) range for indirect assessment of PPO activity of fresh-cut apple slices. Apple slices of two cultivars (cv. Golden Delicious and Elstar) were used to build a robust detection algorithm, which is independent of cultivars and applied treatments. Partial least squares (PLS) regression using the 7-fold cross-validation method and method comparison analysis (Bland-Altman plot, Passing-Bablok regression, and Deming regression) were performed. The 95% confidence interval (CI) bands for the Bland-Altman analysis between the methods were  $-4.19$  and  $13.11$ , and the mean difference was  $3.7e^{-12}$ . The Passing-Bablok regression had a slope of  $0.8$  and an intercept of  $7.6$ . The slope of the Deming regression was  $0.8$  within the CI bands of  $0.56$  and  $1.10$ . These results show acceptable performance and no significant deviation from linearity. Hence, the results demonstrated the feasibility of HSI as an indirect alternative to the standard chemical analysis of PPO enzyme activity.

## 1. Introduction

Processed foods such as fresh-cut and dried apples are becoming more popular due to their nutritional, functional, and convenience properties. However, processed apple products encounter a challenge in meeting the quality demands of consumers [1]. During apple processing, the occurrence of browning reactions can lead to aesthetically unappealing final products. The development of brown pigments during processing is an important criterion in the determination of the quality of fresh-cut apples and dried apple slices [2]. The colour, occurring on the product surface, can be attributed to the activities of polyphenol oxidase

(PPO) and peroxidase (POD) enzymes and the presence of other pigments such as chlorophyll and carotenoids [3, 4]. PPO catalyses two reactions: the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones. These quinones are highly reactive molecules that react enzymatically or nonenzymatically, forming dark brown complex polymers known as melanins [5]. PPO and POD activities are highly dependent on the fruit's maturity, phenolic content, and cultivar [6, 7]. Moreover, they also negatively affect sensory and nutritional attributes of fresh-cut and processed products, thereby reducing consumers' acceptance [8, 9]. Therefore, the food industry has often strived to inactivate oxidative enzymes in order to produce

visually appealing products with a close-to-fresh produce appearance [10].

Several techniques have been extensively investigated to estimate the browning reaction and to control the discolouration of apple slices [11–16]. However, there are limited treatments that could be convenient for small- and medium-scale farmers to inhibit the PPO activity. Additionally, consumers are becoming more health-conscious with a preference for products containing no or a low amount of industrial preservatives. Ascorbic acid (AA), citric acid (CA), hot-water blanching (HWB), steam blanching (SB), and their combinations could be suitable treatments for maintaining a good visual appearance of apple slices [17]. Moreover, these treatments could be affordably utilized by all farmers without extensive technical background.

Previous studies have focused on destructive methods for measuring enzymatic reactions in apples. However, these methods are time- and resource-consuming and make use of chemicals during the analysis with a potential to adversely affect both human health and the environment [5, 18]. In recent years, various techniques for analysing food quality attributes have been developed to replace standard methods. Noninvasive imaging methods, including HSI, have a reputation of being cost-effective in the long term and could eliminate the laboriousness associated with the traditional standard methods [19, 20]. However, the main drawback of HSI is the data dimensionality and size. The resulting images are called data cubes with two spatial dimensions and one spectral dimension at different wavebands, in addition to the signal noise and highly correlated features along the spectral range. The noise and inadequate information can be discarded by handling HSI data which consequently reduces the computational load [21]. HSI has been successfully applied for reliable and accurate measurement of sensory, chemical, and physical properties of foods, such as PPO in mushroom [22], PPO in lychee pericarp [23], moisture content in white button mushroom [24], and moisture content and colour in apple slices [25]. Since PPO exists in multiple forms, its expression may differ among species and cultivars [26]. Algorithms already developed from HSI for mushroom products cannot be directly transferred to other products. Consequently, extensive experiments are required for the development of appropriate algorithms by conducting calibration, validation, and testing.

A robust and accurate statistical analysis is a prerequisite for the proper validation and interpretation of new methods [27]. In many algorithms, the correlation coefficient ( $r$ ) is used to investigate the effectiveness of the model. However, the correlation coefficient ( $r$ ) is a criterion to evaluate the linear association between variables but does not signify a good agreement between the two methods. Therefore, the correlation coefficient ( $r$ ) does not provide confirmation if newly developed techniques perform as well as or even better than standard methods [28]. Ideally, the magnitude of measurement error is known from measurement systems analysis and the guide to the expression of uncertainty in measurement (GUM) [29]. This indicates that measurement systems are subject to certain measurement errors that

require the utilization of special experimental designs. Ordinary least squares (OLS) regression assumes only an error in the  $X$ -direction as it requires known fixed values for  $X$  (independent variable). This approach is not suitable for method comparison, in which an error in the  $Y$ - (dependent variable) direction as well as in the  $X$ -direction must be assumed, since both methods to be compared have a specific known or unknown measurement uncertainty. The minimization of the sum of the squares can, therefore, not be achieved with an ordinary linear regression in the vertical direction. Therefore, statistical approaches such as method comparison are applied to calibrate and validate new methods by assessing repeatability, relative precision, robustness, and reproducibility of the new method [30, 31].

The agreement between methods is deemed good when the paired measures show a high correlation and low random scattering. In the Deming and Passing–Bablok regressions, this is indicated by an intercept which is not confidently different from 0 and a slope which is not confidently different from 1. Furthermore, the error should not be dependent on the measured value and should not have any curvature or any other systematic pattern. The Deming regression considers errors in both  $X$ - and  $Y$ -directions and the optimization is precisely orthogonal to the fitted linear function. The angle of the line depends on the measurement uncertainty in both  $X$ - and  $Y$ -directions [32]. The Passing–Bablok regression is more robust against extreme values or differences (possible outliers) and nonnormal distributed measurement errors [33]. Since each method covers a specific context in which the method comparison is evaluated, both methods are applied. The Bland–Altman plot analysis of differences and agreement offers a visual evaluation between the two methods. Each of the paired measures is represented on the graph by assigning the mean of the two methods the  $X$ -value and the difference between the two methods the  $Y$ -value within the limits of agreement (LOA). The LOA are determined by using the mean and the standard deviation (SD) of the differences between two methods; 95 percent of the data points should lie within  $\pm 2$  SD of the mean difference. However, this does not evaluate whether the agreement is sufficient or suitable to use one method or the other. It simply quantifies the bias (the mean of the differences between the two methods) and the range of agreement. Like the regression approach, LOA shows unsystematic scattering of errors when the methods match well [34].

To the best of our knowledge, no research comparing the HSI method and the standard chemical analysis method in measuring PPO activity in fresh-cut apples has been documented. Moreover, previous studies have only developed algorithms specifically for individual cultivars and treatments. In this study, a robust model that is independent of treatments and cultivars was developed. This study assessed the agreement between HSI and the standard chemical analysis method of determining PPO activity in apple slices of Golden Delicious and Elstar cultivars subjected to several antibrowning treatments. Experimental activities included (1) selecting the most useful VIS-NIR features by using the Variable Importance in the Projection (VIP) plot; (2) developing a model to predict PPO enzyme

activity of apple slice regardless of the cultivar and treatment; and (3) validating the model using method comparison.

## 2. Materials, Methods, and Statistics

**2.1. Samples Preparation.** Two apple cultivars “Golden Delicious” and “Elstar” were purchased from a local supermarket (Göttingen, Germany) and the university farm of the University of Kassel, Hessische Staatsdomäne Frankenhausen (Greibenstein, Germany), respectively. They were visually assessed for uniformity in ripeness and size. The apples were then stored at 4°C in a laboratory refrigerator; afterwards they were conditioned at room temperature before experimental preparations. During the experiments, the apples were washed and the centre cores were removed using 2.5 cm diameter stainless-steel corer (Lurch, Hildesheim, Germany) and sliced to 5 mm thickness using an electrical slicer (Graef, Alleschneider Vivo V 20, Arnsberg, Germany).

A total of 192 apples were used for the experiments, and 576 apple slices were treated with various antibrowning treatments. The experimental design was based upon a split-plot design with 64 runs created using the Design-Expert software version 10 [35]. Before the experiments, three slices were extracted from selected apples. After applying treatments and holding for 60 min at room temperature, hyperspectral images were taken and afterwards PPO activity was analysed. The average value of the three slices was calculated for each run.

**2.2. Antibrowning Treatments.** Both HWB and SB treatments were performed in a temperature-controlled water bath with a maximum capacity of 22 litres (*L*) and interior dimensions of 350 × 220 × 290 mm (Mettert GmbH Co., WNB22, Schwabach, Germany). SB involved injecting steam through a stainless-steel perforated wire tray with stands where the apple slices were placed. The temperatures of water and steam generated in the water bath were measured with a K292 data logger thermometer (Voltcraft, Switzerland). Slices were dipped in hot water of 50°C, 60°C, and 70°C ensuring that the slices were uniformly under the water. The slices were exposed to steam of 65°C, 75°C, and 85°C. After the blanching treatment, slices were either immediately cooled for 2 min at room temperature or directly pretreated by dipping in an acid solution for 3 min at room temperature; then the residual PPO enzymatic activity was evaluated. Dipping treatments were performed using either 1% (w/v) AA or 1% (w/v) CA or a combination of both solutions. After treatment, the apple slices prepared for use as control samples and treated samples were exposed to ambient temperature for 60 min after the slicing operation (e.g., control slice (a), slice immediately after treating with 70°C HWB + 1% AA + 1% CA (b), and 70°C HWB + 1% AA + 1% CA treated slice exposed for 60 min at room temperature (c)), as presented in Figure 1.

**2.3. Hyperspectral Imaging, Image Segmentation, and Preprocessing.** The HSI system included a camera mod.

V10 E PFD (Specim Spectral Imaging Ltd., Oulu, Finland) coupled with a Schneider 35 mm lens (Xenoplan 1.9/35, Schneider Optische Werke GmbH, Bad Kreuznach, Germany) and a linear translation stage (Specim Spectral Imaging Ltd., Oulu, Finland). The camera captures slice images in the visible (VIS) and the near-infrared (NIR) range between 400 and 1000 nm at 1.5 nm increments. Three 60 W halogen GU10 bulbs illuminated the platform on which the slices were placed. During scanning, the translation stage moved at a speed of 8 mm s<sup>-1</sup> and the exposure time was 20 msec. The distance between camera lens and the surface of apple slices was set at 270 mm. Three slices per experiment were scanned. Each complete image consisted of those three slices and the white reference tile. The apple samples were saved in the ENVI 3.6 format (Research Systems, Inc., USA) for further processing.

The hyperspectral images were calibrated with both white and dark references. These references were acquired to correct the raw images by removing random noise, unevenness in the light source intensity and to normalize the output of the camera. The white reference consisted of a ceramic tile of 200 × 24 mm with an overall image spatial resolution of 1632 × 1302 pixels. The dark reference was obtained by closing the shutter of the camera.

The relative reflectance spectrum for each pixel in the image ( $R(\lambda_{xy})$ ) was calculated using the following equation with a spatially averaged white reference illumination spectrum  $W(\lambda_{xy})$ , dark reference image ( $D\lambda$ ), and a sample irradiance spectrum  $S(\lambda_{xy})$ :

$$R(\lambda_{xy}) = \frac{S(\lambda_{xy}) - D\lambda}{W(\lambda_{xy}) - D\lambda} \quad (1)$$

The average relative reflectance spectrum for each sample was then calculated automatically.

Hyperspectral preprocessing methods, such as normalization, multiple scattering correction (MSC), and the first derivative, were executed to improve original spectra by eliminating noise and extracting informative spectral variables before model development [36]. In this study, normalization was used to scale the data to ensure each input parameter (pixel) has a similar data distribution and average values.

Image segmentation, background and dead pixel removal, noise removal, and average reflectance calculation were carried out using MATLAB 2015a (The MathWorks Inc., Natick, MA, USA), following the methods used in a previous study [25].

**2.4. Colour Assessment.** The surface colour of the apple slices was measured with a CR-400 Chroma Meter (Minolta, Osaka, Japan). The measurements were based on the CIE Lab colour space with the CIE Standard Illuminant C and 2-degree observer angle. The colorimeter was calibrated using a standard white reflector plate with values of  $Y$  (92.4),  $x$  (0.316), and  $y$  (0.3322). Four replications were carried out for each sample by performing two colour measurements on each side of the slices. The results were expressed in terms of luminance ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) [37].

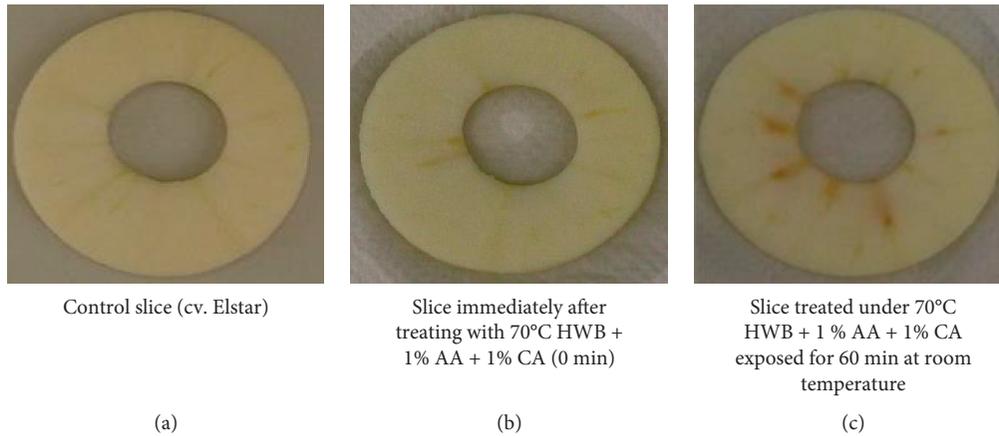


FIGURE 1: Illustration of the appearance of fresh-cut apple slices at different conditions: (a) control slice; (b) slice immediately after treating with 70°C HWB + 1% AA + 1% CA; and (c) 70°C HWB + 1% AA + 1% CA treated slice exposed for 60 min at room temperature.

## 2.5. PPO Enzyme Activity (Wet-Chemistry Method)

**2.5.1. Enzyme Extraction.** Enzyme extraction was carried out in accordance with Massantini et al. [38]. Ten grams of apple samples were homogenized with 20 mL of chilled sodium phosphate buffer (PBS, 0.1 M, pH 6.5) containing 1.5% (w/v) polyvinylpyrrolidone and 1% (v/v) Triton X<sub>100</sub>. The homogenization was performed under ice-cooling conditions using an Ultra-Turrax T25 (IKA Instruments Ltd., Staufen, Germany). The mixture was centrifuged at 12,400 × g for 20 min at 4°C using a centrifuge mod. Eppendorf 5416 (Sigma-Aldrich, Darmstadt, Germany). The supernatant was then filtered through a Macherey-Nagel (MN) 640 w filter paper. Finally, the crude enzymatic extract was stored at -80°C until use.

**2.5.2. Enzyme Activity Measurement.** PPO activity was measured by following the method developed by Moschetti et al. [39] with a slight modification. The assay was performed using a spectrophotometer mod. HP 8453 UV-Vis system (Agilent Technologies GmbH, Waldbronn, Germany). Catechol (Sigma-Aldrich, St. Louis, Missouri, USA) was used as a substrate. The final reaction mixture contained 1.5 mL of catechol (40 mM), 2.3 mL of PBS (0.1 M, pH 6.5), and 0.2 mL of crude enzyme. Changes in the absorbance at 420 nm were monitored for 2 min upon oxidation of the substrates catalysed by the enzyme. One unit of enzyme activity (U) was defined as an increase in absorbance of 0.001 min<sup>-1</sup>. Enzyme activity was measured in duplicate.

**2.6. Statistical Analysis, Modelling Technique, and Method Comparison.** Partial least squares (PLS) regression and principal component analysis (PCA) are the common methods for multivariate analysis in the field of chemometrics. These methods help to analyse a set of dependent variables from a large set of independent variables or predictors [40]. Models are built by extracting successive orthogonal latent factors that maximize the covariance structure between the response and predictor variables in a

robust way. PCA is then performed on the preprocessed spectra collected from all samples. PCA uses orthogonal transformation to convert highly dimensional and highly correlated data into linearly uncorrelated variables known as principal components (PCs). A particular original wavelength variable has a specific loading on each PC. High loading indicates high importance of a specific wavelength on a PC. PCs explain the descending importance of explainable variation in datasets. Loadings of the first few PCs with the greatest contribution are used to identify important wavelengths [41, 42].

Based on the waveform and multicollinearity of the hyperspectral data, the following steps were performed for regression model development: (1) PCA was carried out before developing the calibration model to visualize any relevant and interpretable structure in the data and to detect the outliers. PCA was also performed to correlate specific quality attributes such as water content, colour, and PPO in the wavebands in accordance with the literature [21, 43]; (2) regression model development was conducted using the full spectrum with PLS. Fully cross-validated PLS models were developed to predict PPO activity. The optimum number of latent factors was used to build a robust PLS model to obtain the best prediction performance. The optimum number of PLS-latent variables for each feature and the number of factors that produced the least predicted residual errors sum of squares (PRESS) were selected as the optimum values [44]. These optimum factors were selected to guard against over- and underfitting problems and to model the variability in the data. Due to the limited number of observations, the data was not split into a training and validation subsets. However, the regression model was cross-validated using the 7-fold cross-validation (CV) method [45, 46].

Additionally, the Variable Importance in the Projection (VIP) was used to select the important wavelengths from the PLS models [31, 47]. The VIP scores obtained by PLS regression can be used to select the most influential variables or predictors,  $x$ . The  $j$ th  $x$ -variable can be estimated by the following equation:

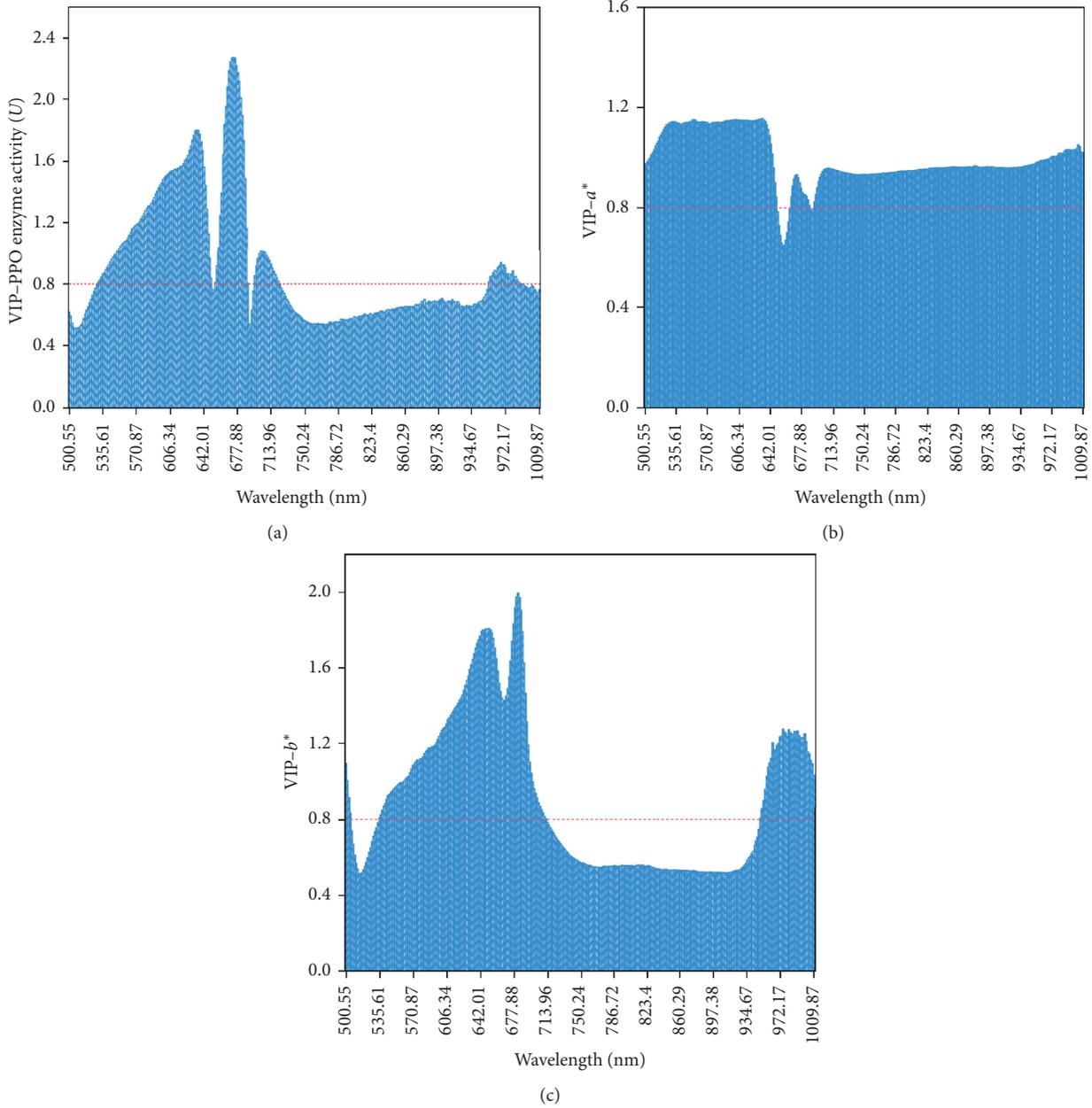


FIGURE 2: VIP plot for PPO activity (a),  $a^*$  (b), and  $b^*$  (c) as a function of wavelength.

$$V_j = \sqrt{P} \sum_{a=1}^A SS_a \frac{(W_{aj}/W_a)^2}{SST}, \quad (2)$$

where  $P$  is the total number of variables,  $A$  is the total number of components,  $SS_a$  is the sum of squares explained by the  $a^{\text{th}}$  component,  $SST$  is the total variance explained by all the components, and  $(W_{aj}/W_a)^2$  is calculated using loading weight vectors  $W_{aj}$  for each component and represents the importance of the variable  $j$  for component  $a$ . The predictor variable whose VIP score is greater than 0.8 is considered as an important variable in this study considering the average of squared VIP score [48].

In this study, PLS was applied to find linear relationships between hyperspectral features and PPO activity through a nonlinear iterative partial least squares (NIPALS) algorithm.

Statistical analysis for method validation was done by Passing–Bablok regression [49], Deming (orthogonal in case error ratio = 1) regression [50], and the Bland–Altman plot [51].

Due to the low signal-to-noise ratio at the lower end of the spectral range, only the wavebands in the spectral range from 500 to 1009 nm were used to evaluate the data. Both apple cultivars and all treatments were used to develop the PLS regression model and for method validation. The basic assumption was that the measurement methods were

independent of the sample treatment and apple cultivar for the purposes of model robustness [52].

Data handling and statistical analysis were both performed using JMP Pro 14.2.0 software (SAS Institute Inc., Cary, NC, USA).

### 3. Results

#### 3.1. Prediction of Polyphenol Oxidase Activity

**3.1.1. Selection of Wavebands from VIP Plot.** As shown in Figures 2(a)–2(c), the PPO,  $a^*$ , and  $b^*$  parameters have VIP scores greater than 0.8. The wavelength around 677 nm was found to be important for the determination of PPO activity. The development of brown colour at approximately 677 nm is also noticeable in the  $a^*$  and  $b^*$  parameters as shown in Figures 2(b) and 2(c).

**3.1.2. Characteristics of Spectral Profiles of Apple Slices.** The average reflectance spectra extracted from regions of interest (ROIs) of apple slices treated with different treatments are shown in Figure 3 in the wavelength range of 500–1000 nm. A similar overall trend throughout the whole wavelength range was observed for all samples.

The wavelength at around 677 nm was found to be an interesting absorption region, which was as well as that observed from the VIP plot. The changes in the spectral profiles for slices treated with different treatments showed similar spectral curves and slight differences in reflectance values. For instance, Elstar slices treated with HWB 60 + 1% AA + 1% CA showed nearly no spectral patterns, which might be due to the inactivation of PPO activity. This might be due to the loss of main secondary structure elements of PPO mainly during the thermal treatment [53].

#### 3.2. Comparing the Methods of Measurement

**3.2.1. Regression Analysis to Check Bias between Methods Using the Intercept and the Slope as Indicators.** PLS regression analysis was performed using 4 latent variables provided by the minimum root mean PRESS (Figure 4(a)). The comparison between measured (reference laboratory method) and predicted values of PPO activity by PLS is shown in Figure 4(b). A strictly paired dataset was built by the laboratory-measurement values as  $X$ -variable and the predicted values by HSI as  $Y$ -variable for the method comparison analysis. Both the Deming regression (red line) and the Passing–Bablok regression (blue line) were performed.

It can be observed that the values obtained from the reference chemical method and the predicted values from the PLS model are randomly scattered around the line of equality (Figure 4(b)). The slope of the Deming regression was 0.80 and that of the Passing–Bablok regression was 0.81, both of which are not confidently different from 1. The intercept of the Passing–Bablok regression was 7.62, which is also not confidently different from 0 (Table 1). The orthogonal fit ratio was found to be 0.64 (Table 2).

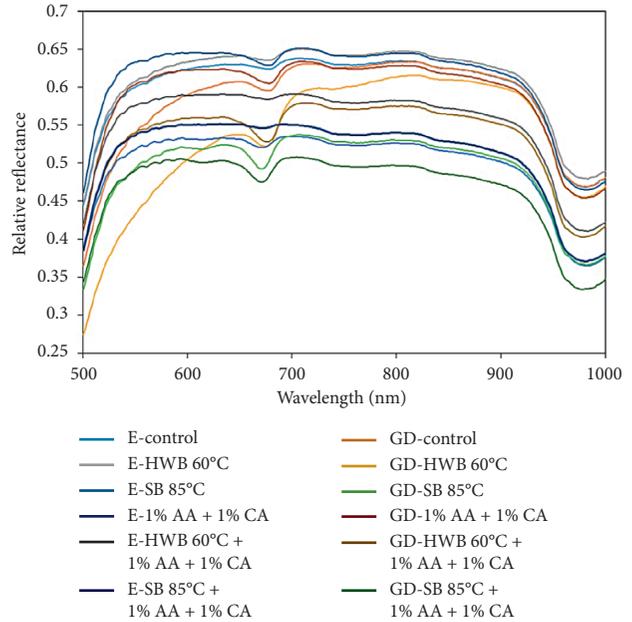


FIGURE 3: Spectral profile of apple slices.

**3.2.2. The Bland–Altman Plot to Check General Agreement Instead of Correlation between Methods.** The Bland–Altman plot analysis of differences and agreement is provided in Figure 5. The plot was constructed by plotting the difference between the predicted data from PLS and the measured PPO activity against the mean of both methods. The plot displays an acceptable agreement between noninvasive HSI technique and the chemical method (95% CI:  $-22.4$  to  $22.4$ ) (Table 3). As shown in Table 4, the mean difference (mean bias) was found to be  $3.7e^{-12}$  with a standard deviation (SD) of 11.43.

### 4. Discussion

The suitability of noninvasive measurement using HSI for detecting PPO activity on fresh-cut apple slices was investigated by comparison with the standard chemical analysis method. According to Mollazade [54], the most suitable wavelength range for the determination of PPO activity in Button mushroom was 470–640 nm. In this study,  $X$ -variables (spectral wavelengths) with a VIP score greater than 0.8 were found to be important for modelling the metric ( $Y$ -variables). Around 677 nm, a VIP score plot greater than 0.8 indicated that the pigment changes such as chlorophyll a were perhaps related to PPO and brown colour development [55]. This could also be due to functional pigments (i.e., chlorophyll a and the accessory pigments) which absorb at wavelengths shorter than 700 nm [56, 57]. Jian et al. [58] used the visible and shortwave near-infrared (VIS/SW NIR) technique in the range 400–1100 nm to evaluate the bruising susceptibility in Golden Delicious apple. Their findings showed a change in colour as a result of chlorophyll content at around 680 nm during prediction of the bruising of apples at maturity stage. Other studies have also shown that PPO activity leads to browning reactions [5, 14, 23, 59]. Therefore,

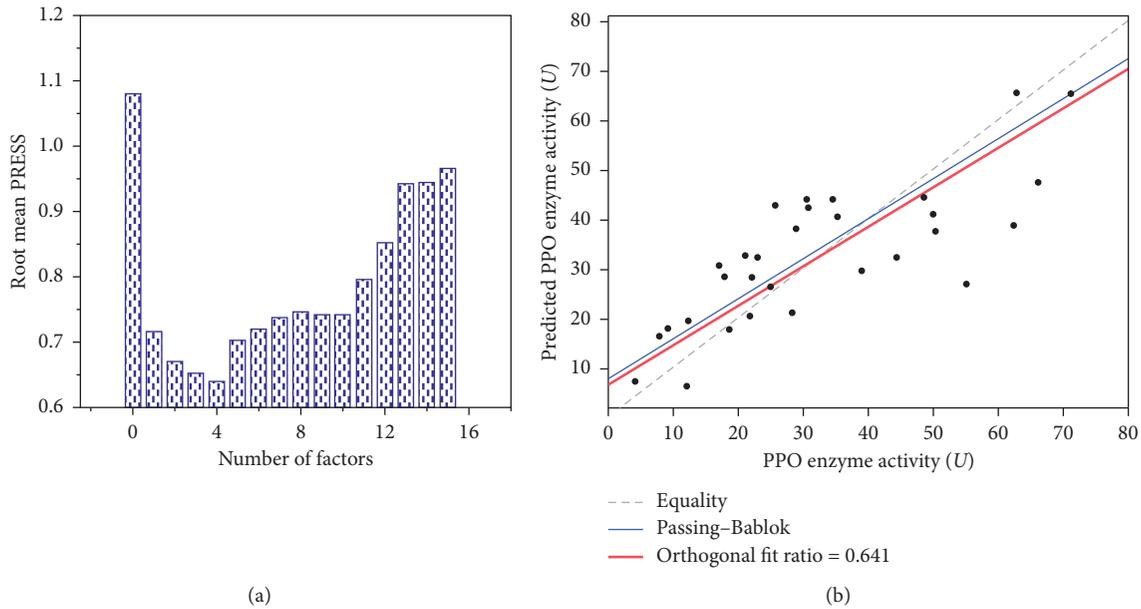


FIGURE 4: (a) RMSE PRESS plot for PPO as a function of the number of factors. (b) Method comparison-regression analysis: regression analysis: PPO predicted by PLS vs. PPO measured by the gold standard method. The dashed grey line: a line of equality, solid red line: result of the Deming regression with the obtained fit ratio, and solid blue line: a line of Passing-Bablok regression.

TABLE 1: Analysis details of the Passing-Bablok regression.

Parameter	Passing-Bablok regression		
	Estimate	Lower CL	Upper CL
Intercept	7.620	-39.774	30.550
Slope	0.809	0.039	2.683
Tau	0.612	—	—

TABLE 2: Analysis details of the orthogonal regression.

Parameters	Orthogonal fit ratio = 0.641	
	PPO 60	Predicted PPO 60
Mean	31.695	31.695
Std. deviation	19.067	15.261
Variance ratio	0.640	
Correlation	0.800	
Intercept	6.325	
Slope	0.800	
Lower CL	0.597	
Upper CL	1.072	
Alpha	0.050	

the results from this study are in agreement with previous studies indicating relative reflectance spectra at 677 nm.

The mean and the standard deviation of differences between the predicted and measured PPO obtained in this study were in agreement with the statistical limits defined by Bland and Altman [51]. However, a full-scale measurement system analysis could not be applied as this study only assessed whether the HSI method can be used as an alternative to the standard chemical analysis method. Consequently, a real measurement system analysis is necessary to confirm the repeatability and reproducibility of the HSI

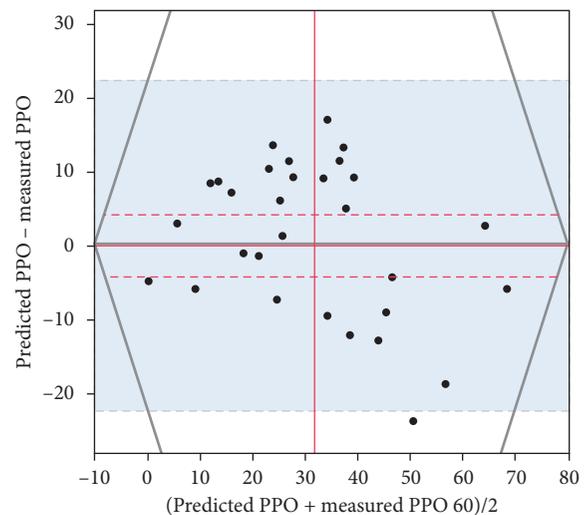


FIGURE 5: Method comparison—Bland-Altman plot: (a) Bland-Altman plot: the differences between PPO predicted and PPO measured vs. the mean value of both measurements. Dashed grey lines indicate the limit of agreement ( $0 \pm 1.96$  SD of difference), the solid black line is the line of equality, the solid red line shows the mean difference (bias), and the dashed red lines show the 95% CI around the mean difference.

TABLE 3: Descriptive statistics from the Bland-Altman analysis.

Parameter	Bland-Altman		
	Estimate	Lower CL	Upper CL
Std. deviation	11.4295	8.5376	14.3216
Upper limit	22.4019	15.2369	29.5668
Lower limit	-22.402	-29.567	-15.237

TABLE 4: Mean difference between methods and correlation analysis from the Bland–Altman analysis.

Parameter	Value	Parameter	Value
Mean difference	$3.7 \times 10^{-12}$	<i>t</i> -Ratio	$1.8 \times 10^{-12}$
Std. error	2.0528	DF	30
Upper 95%	4.1923	Prob >   <i>t</i>	1.0000
Lower 95%	-4.1924	Prob > <i>t</i>	0.5000
<i>N</i>	31	Prob < <i>t</i>	0.5000
Correlation	0.8004		

method in a robust setup [29]. Two independent methods measuring the same variable might have their own inherent error. The dispersion observed in Figure 3 could be due to physical and chemical heterogeneity of the sample which is indicated by outliers outside the LOA [60]. Additionally, critical patterns in the plots indicated constant bias as well as slopes and curves (nonconstant bias) or variance heterogeneities [51, 60]. The orthogonal fit ratio of 0.64 proved that both measurement methods had different measurement uncertainties. Nevertheless, the two approaches were found to be comparable since the mean difference is close to zero and the standard deviation of the differences between measured values reveals no systematic variation from the mean of the measurement pairs. Furthermore, the standard deviation of difference between the noninvasive and invasive methods was significantly small showing an acceptable precision, and thus the two methods can be used interchangeably to predict the enzyme activity.

## 5. Conclusions

The investigation on apple PPO activity is a fundamental challenge for the food industry and to the researchers. Proper control of browning reactions prevents adverse effects on the visual appearance of foods as perceived by the final consumer. This study investigated the suitability of HSI as a noninvasive method for the measurement of PPO on fresh-cut apples. Results showed that the changes in PPO activity of slices were mainly at wavelengths around 677 nm as is indicated by the VIP plot and spectral profile. A robust algorithm that is independent of both cultivars and applied treatments was developed. For this purpose, method comparison (Bland–Altman, Passing–Bablok, and Deming regressions) was conducted to assess HSI as an alternative method to the standard chemical method. Thus, the study clearly indicates that PPO measurement using HSI is applicable for automated real-time and in-line measurement.

This study provides an important first step towards the adoption of HSI as a nondestructive method of establishing enzyme activity in apples irrespective of cultivar. In our case, 7-fold cross-validation technique was performed, which makes the chance of overfitting much lower by striking a balance between modelling the intrinsic structure of the data and modelling the noise. Moreover, several hundred samples for both calibration (training) and validation sets are sufficient at the proof-of-concept stage. However, the technique should be validated more extensively before implementation

in industry. Thus, the data is essential for evaluating model performance using independent data to assess the suitability of the developed models.

Further research and in-depth analysis of the spectral changes and chemometrics of enzyme activity in apple slices from different cultivars will be needed to develop a more precise and robust model.

## Data Availability

The data used to support the findings of the study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Authors' Contributions

Luna Shrestha, Boris Kulig, and Barbara Sturm conceived and designed the experiments. Luna Shrestha performed the whole experiments and was the lead author of the manuscript. Luna Shrestha and Boris Kulig analysed the data and the interpretation of the results. Boris Kulig and Roberto Moschetti helped in structuring the manuscript and the interpretation of the results. Barbara Sturm also supported in terms of improvement of the manuscript style and content as well as English language. Elke Pawelzik provided all types of facility and supervision to conduct the laboratory work. Riccardo Massantini, Elke Pawelzik, and Oliver Hensel contributed their critical comments to improve the manuscript.

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