An examination of the principle of non-destructive flesh firmness measurement of peach fruit by using VIS-NIR spectroscopy

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Abstract

Evaluating the maturity of peach fruit is desirable during both the preharvest and postharvest periods, and flesh firmness (FF) is a representative maturity index. Although a non-destructive FF measurement technique using visible (VIS) and near-infrared (NIR) spectroscopy has been developed, the principle has been unclear. This study was conducted to examine the structure of the FF prediction model by comparing with that of the model for measuring water-soluble pectin (WSP) content. Those two prediction models have the same information regions related to the colors of pericarp and mesocarp (chlorophyll) and to a water band in the NIR region. Moreover, a statistical heterospectroscopy analysis between NIR and $^1$H nuclear magnetic resonance (NMR) spectra suggests the possibility that absorptions of methanol and succinate as well as galacturonic acid embedded in a water band play important roles in predicting FF. This approach would enhance the reliability of nondestructive VIS-NIR prediction models in many practical situations.

Keywords: Food science, Analytical chemistry
1. Introduction

On-tree evaluation of the maturity of peach fruit is desirable because if they are harvested in an immature state, the fruits would never attain a quality acceptable for consumption. Because melting-type peach fruit harvested in a ripened state rapidly softens during the postharvest period, they are usually harvested at an early stage of ripening so that they attain a superior eating quality after reaching the consumers. On their part, consumers and retailers need to judge as to when a fruit can be eaten, by subjectively evaluating the degree of maturity based on the ground color and flesh firmness (FF). Therefore, evaluating the maturity of fruit is also important during the postharvest period.

While peach fruit matures, its color changes from green to red or yellow due to a reduction in chlorophyll content. Additionally, FF softens, flavor intensity increases, sucrose content increases whereas fructose and glucose contents decrease, total acid content decreases mainly because of the loss of malic acid, and respiration and ethylene production increases [1]. Ethylene is a plant hormone that plays a crucial role in the initiation and continuation of ripening of climacteric fruits such as peaches, apples, and bananas [2]. Sensory attributes, such as the intensity of flavor, sweetness, and fruitiness also increase during ripening [3]. Among these maturity indices, the ground color is considered the most practical and reliable index for deciding whether or not the fruit has reached the proper minimum maturity stage for harvest, and FF can be used as a maximum maturity index to ensure the retention of good quality after shipping [1].

The ground color of peach fruit on a tree is generally evaluated based on visual assessment by experts. Costa et al. [4] introduced a non-destructive optical technique for evaluating the chlorophyll content in fruit mesocarp, which is an index of absorbance difference ($I_{AD}$), calculated as the difference between the absorbance at 670 nm (near the chlorophyll-a absorption peak) and 720 nm (the background of the spectrum). Because $I_{AD}$ relates to the actual chlorophyll-a content during fruit ripening, it allows for the determination of the optimal harvest time.

FF has been measured traditionally via a destructive method based on the penetration of a cylindrical plunger into the flesh of a peeled fruit. A non-destructive FF measurement technique has been studied using various technologies [5] such as impact techniques (micro-deformation [6, 7, 8], impact sensor [9, 10, 11], and acoustic response [12, 13]) and optical techniques [14, 15, 16]. The impact techniques are more accurate and reliable. However, the optical techniques have the advantage that they do not require direct contact with the fruit and can simultaneously estimate several other internal characteristics, including sugar content and internal defects. The drawbacks of the optical techniques include poor accuracy and unclear principle. In-line optical devices based on near-infrared (NIR) spectroscopy that claim the
ability to evaluate “ripeness” have been developed by some companies, including SACMI (Italy), AWETA (The Netherlands), GREEFA (The Netherlands), and Com-pac (New Zealand). However, the definition of “ripeness” is obscure and the understanding of the principles on which these techniques are based is very limited because of the generally proprietary nature of these techniques. Although the detailed mechanisms of fruit softening are still not clear, it is generally understood that cell wall-modifying enzymes are involved [17] and that water-soluble pectin (WSP) increases during ripening in the melting type peach owing to the progress of pectin hydrolysis [18]. FF is a characteristic of fruit tissue structure, but WSP is a saccharide in fruit and should absorb radiation of any wavelength. Therefore, we have a hypothesis where the information related to WSP is used to develop a FF prediction model via NIR spectroscopy.

In this study, to specify an appropriate index for NIR evaluation of the maturity of fruit in the postharvest period, various indices; ethylene production, respiration, FF, soluble solid content (SSC), and WSP content of peaches for eight days after harvest were compared. Nondestructive evaluation of fruits by visible (VIS)-NIR spectroscopy is known as a mature technology [19], however, it has been empirically given by regression analyses. We examine the working mechanism of the prediction models for FF, WSP content, and elapsed days with the aid of 1H nuclear magnetic resonance (NMR) spectroscopy, which gives both of qualitative and quantitative signal features.

2. Materials and methods

2.1. Plant materials

A total of 40 melting type peaches (“Akatsuki” [Prunus persica L. Batsh], grown in the experimental farm at the Institute of Fruit Tree and Tea Science, NARO, Japan) were harvested from a single tree at one time, at their commercial harvest maturity stage as assessed by an expert based on the ground color, fruit size, and FF. The fruits were next ripened at room temperature (approximately 22–25 °C). Five fruits were sampled each day from the harvest date (first day) to the eighth day, and the fruit weight, ethylene production, respiration (O₂, CO₂, N₂), VIS-NIR spectra, FF, SSC, and WSP content were measured. To monitor changes in the same fruit, the five fruits to be sampled on the last day (referred to as the “monitoring samples”) were used daily for measurements of the non-destructive maturity indices: fruit weight, ethylene production, respiration, and VIS-NIR spectra, and returned to the storage room daily. The experimental design is illustrated in Supplementary material (Fig. S-1).
2.2. Ethylene production, respiration, VIS-NIR spectra, flesh firmness, and soluble solid content

Ethylene production and respiration were measured via gas chromatography system GC-14B (Shimadzu Corporation, Kyoto, Japan). The fruit was placed in a sealed jar and left at room temperature for 1 h. A 0.5-mL gas sample was then injected into the GC system.

The VIS-NIR spectra of the intact fruit were measured in the wavelength range of 500–1000 nm (at 2 nm intervals) using a commercial portable spectrometer K-BA100R (Kubota Corporation, Osaka, Japan) with interactance mode (See Fig. 1). The incident light from the tungsten-halogen lamp reached the sample via a fiber-optic bundle ring with 38 mm diameter and penetrated the fruit. The light was conducted through the fruit by repeated transmission, reflection, absorption, and scattering, and consequently a portion of diffusely reflected light was sent to the detector via another fiber-optic bundle with 5 mm diameter set at the center of the illuminator ring. The spectra were measured on two opposite sides at the equatorial region, specifically 90° to the right and left of the suture line, at room temperature. The integration time for each acquisition was 90 ms.

A Magness-Taylor-type fruit penetrometer FT011 (Effegi, Florence, Italy) with an 8-mm cylindrical plunger was used for the FF measurement. The FF value was measured on the same site as was used for the spectral measurement, after peeling the epicarp. The part of the mesocarp at the FF measurement site was hollowed out and squeezed for SSC measurement with a digital refractometer PAL-BX/RI (ATAGO Co., Ltd., Tokyo Japan). A portion of fruit hollowed out from the upper part of the spectral measurement site (90° to the right of the suture line) was then frozen in liquid nitrogen and stored at −30 °C until used for WSP measurement.

Thereafter, juice was squeezed from the whole fruit and then frozen in liquid nitrogen and stored at −30 °C until use for the NMR measurement with AVANCE 500 MHz equipped with a CryoProbe (Bruker Optics GmbH, Ettlingen, Germany).

![Fig. 1. Measurement of VIS-NIR spectra of the intact fruit with a commercial portable spectrometer.](https://doi.org/10.1016/j.heliyon.2018.e00531)
2.3. Water-soluble pectin content

The cell wall polysaccharides were prepared and fractionated as described by Yoshio et al. (2011) [20], using the method based on that of Huber and O’Donoghue (1993) [21]. Briefly, 2 g of frozen flesh was homogenized in a 50-mL centrifuge tube with 8 mL of ice cold 0°C ethanol using a homogenizer AHG-160A (AS ONE Corporation, Osaka, Japan) and centrifuged at 1600 g for 10 min. The insoluble pellet was washed twice with 10 mL cold 80 % ethanol, treated with 2 mL Tris-buffered (pH 8.0) phenol for 1 h at room temperature, and precipitated with 10 mL of ethanol at −30 °C. The ethanol precipitate was extracted with 10 mL of chloroform/methanol (1:1, v/v) for 30 min at room temperature, and washed once with 10 mL acetone. The precipitate was collected at each step by centrifugation at 1600 g for 10 min. The alcohol-insoluble solid (AIS) was incubated with 10 mL water at room temperature for 12 h. The supernatants were used for the water-soluble extract. The content of galacturonic acid (GalUA) in the extract was determined using the m-phenylphenol colorimetric method [22]. The absorbance of the solution at 520 nm was measured with SolidSpec-3700DUV (Shimadzu Corporation, Kyoto, Japan).

2.4. NMR analysis

The NMR spectra of juice samples were measured after thawing and dilution with a deuterated phosphate buffer solution (pH 7.0). One- and two-dimensional NMR spectra were acquired processed as described previously [23]. Metabolite signals were annotated primarily using the SpinAssign program from the PRIME web service [24, 25]. HSQC data were processed and the peak table was prepared using NMRPipe and NMRDraw [26]. GalUA signals were unequivocally assigned by 2D spectra and spiking experiments.

3. Results and discussion

3.1. Time-dependent changes in the maturity indices

The transitions of the non-destructive maturity indices for five monitoring samples are depicted in Fig. 2. The monotonic decrease in the fruit weight may be the result of reduced water content. N2 and O2 production both increased monotonically and CO2 production also showed an increasing trend. Ethylene production increased from the third to the sixth day and then slowly decreased after the peak. Since a SSC prediction model developed by Kubota Corporation was pre-installed in the K-BA100R spectrometer, the predicted SSC values were obtained from the intact fruit. The predicted SSC gradually increased during the experimental period. The reliability of the installed SSC prediction model was verified by comparing the predicted values with the digital refractometer values for all 40 samples (r = 0.94).
The statistics of the maturity indices for 40 fruits (5 fruits sampled each day) are shown in Table 1. Variations in the mean value are shown in Fig. 3. The monotonic decrease in weight and increases in O$_2$, N$_2$, and SSC, as in the case of the monitoring samples, were not observed. The time-dependent changes in weight, respiration, and SSC within an individual fruit were not more significant than the changes between fruits. In contrast, the variation in ethylene production was similar to that in the monitoring samples and peaked on the fourth day. The FF value showed a steep decline from the first to the third day and then decreased slowly. The WSP content rapidly increased from the second to the third day and then showed a gradually

![Fig. 2. Time-dependent changes in the non-destructive maturity indices for five monitoring samples.](image)

### Table 1. Statistics of the maturity indices.

| Index               | Min   | Mean  | Max   | SD   |
|---------------------|-------|-------|-------|------|
| Ethylene (ng kg$^{-1}$ s$^{-1}$) | 0.17  | 3.24  | 8.24  | 1.80 |
| N$_2$ (mg kg$^{-1}$ s$^{-1}$)      | 1.59  | 2.90  | 3.87  | 0.62 |
| O$_2$ (mg kg$^{-1}$ s$^{-1}$)      | 0.23  | 0.43  | 0.57  | 0.09 |
| CO$_2$ (mg kg$^{-1}$ s$^{-1}$)     | 0.021 | 0.025 | 0.032 | 0.002|
| pH                                | 4.00  | 4.48  | 5.00  | 0.22 |
| SSC (%)                           | 9.5   | 12.6  | 17.8  | 1.7  |
| FF (N)                            | 5.29  | 13.90 | 39.98 | 8.10 |
| WSP in AIS (g kg$^{-1}$)           | 36.34 | 163.22| 267.14| 64.26|

SD: standard deviation, SSC: soluble solid content, FF: flesh firmness, WSP: water-soluble pectin, AIS: alcohol-insoluble solids.
increasing trend. The FF and WSP content were negatively correlated \((r = -0.81)\). Consequently, we confirmed that the FF and WSP content were useful for evaluating the maturity during the postharvest period because these values gradually changed in one direction, unlike ethylene production.

### 3.2. VIS-NIR spectra

The raw spectra of the intact fruit are shown in Fig. 4a. The spectra were averaged for each day, and the line color gradually darkened with the day. Owing to the pigments
in the mesocarp and pericarp, strong absorption was observed in the visible region (500–700 nm). Absorption of chlorophyll-α was observed around 670 nm and its intensity gradually declined during ripening. A baseline shift, depending on the progress of ripening, was found in the 700–1000 nm wavelength range. The baseline correction using the asymmetric least squares method [27] revealed a gradual decrease of strong absorption with a peak at 970 nm (Fig. 4b), mainly due to water. As mentioned above, the time-dependent decrease in fruit weight was not observed in these samples (Fig. 3); hence, factors other than the reduced water content resulted in the gradual decrease in the absorption.

3.3. PLS regression models for estimating the FF and WSP content

The prediction models for measuring FF and WSP content were developed by applying the partial least squares (PLS) regression to the spectra, using a custom-designed program written in R (The R Foundation for Statistical Computing, Vienna, Austria) with the “pls” package [28]. Savitzky-Golay smoothing [29] (windows size = 11 points, polynomial order = 2) was applied to the spectra because the signals at both ends of the VIS-NIR spectra were slightly noisy due to the detector efficiency. The WSP prediction model was built using only the spectra measured on the right side of each peach because the WSP content was determined using a portion of fruit at the spectral measurement site on the same side. In addition to FF and WSP, we also built a prediction model for measuring the elapsed days (ED) from the harvest for Fig. 4. (a) Raw and (b) baseline corrected spectra of the intact peach fruit. The spectra were averaged for each day and the line color gradually darkened with each day.
The ED prediction model was built using the spectra generated by averaging the spectra taken on the right and left sides of each fruit.

The statistics of the PLS regression models built with the smoothed spectra are shown in Table 2. Cross-validation was performed using the Venetian blinds method with ten segments instead of prediction test with a separate dataset. The determination coefficients ($R^2$) were 0.80, 0.82, and 0.90 for FF, WSP, and ED, respectively. Root mean squared error of cross-validation (RMSECV) is normally slightly larger than root mean squared errors of calibration (RMSEC), whereas RMSECVs in Table 2 show more than 20 percent increases from RMSECs. This result is attributed to the small sample size. For the same reason, RPD values (the ratio performance of standard deviation to standard error of cross validation) for the models are on a borderline (RPD = 2) of nonreliable models and fair models. The models given here are not suitable for practical use, however, we are going to discuss the qualitative aspects of the correlation between VIS-NIR spectra and FF, WSP, and ED. The scatter plots of the measured value and the predicted value are listed in Fig. S-2. Although the spectral preprocessing methods, such as standard normal variate, first and second derivative, auto-scaling, and their combinations were examined, they did not improve the performance of the models. This indicates that scattering profile, which varies depending on the sample tissue structure, is also useful for developing these three models as Lu et al. (2005) showed the correlation between scattering profiles and FF [30]. The profiles of the regression vectors for these three models were quite similar (the difference is just the sign), and the informative bands were common according to variable importance in projection [31] (threshold: VIP >1), as shown in Fig. 5. The absorption bands in the wavelength ranges 500–560 and 630–690 nm were related to the pigments, especially chlorophyll. The wavelength range of 930–1000 nm corresponds to the water band, where the absorption gradually decreased during the experimental period, as shown in Fig. 4b. We can conclude that these models are based on the same information that changes during ripening.

### 3.4. NIR-1H NMR statistical heterospectroscopy

It is extremely difficult to assign the broad, ill-defined, and overlapping absorptions in the NIR spectra. A correlation analysis with the measured concentration of each Table 2. Statistics of the partial least squares (PLS) regression models.

| Index       | N  | LVs | $R^2$ | RMSEC | RMSECV | Bias | RPD |
|-------------|----|-----|-------|-------|--------|------|-----|
| FF (N)      | 80 | 9   | 0.80  | 3.60  | 4.41   | 0.07 | 1.67|
| WSP in AIS (g kg⁻¹) | 40 | 9   | 0.82  | 27.08 | 41.16  | −2.50| 1.31|
| ED          | 40 | 10  | 0.90  | 0.70  | 1.05   | −0.05| 2.03|

N: number of samples, LVs: number of latent variables, $R^2$: determination coefficient of calibration, RMSEC: root mean squared error of calibration, RMSECV: root mean squared error of cross-validation. Bias: mean error of calibration, RPD: ratio performance deviation, FF: flesh firmness, WSP: water-soluble pectin, AIS: alcohol-insoluble solids, ED: elapsed days.

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component in the sample is one possible solution. To measure various components at once, $^1$H nuclear magnetic resonance ($^1$H NMR) spectroscopy was adopted as a realistic and convenient technique. We then conducted the correlation analysis between the NIR spectra showing absorption in the range of 930–1000 nm and the $^1$H NMR spectra obtained from the juice of the same samples. The correlation analysis between different kinds of spectroscopic datasets was named “statistical heterospectroscopy (SHY)” by Nicholson’s group [32]. They originally applied SHY to NMR spectroscopic datasets and mass spectrometric datasets to identify molecular biomarkers. Graça et al. (2013) demonstrated the mid-infrared (MIR)-NMR SHY to find biomarker MIR signatures for prenatal disorders [33]. In this study, we applied SHY to NMR and NIR spectroscopic datasets for understanding the NIR prediction model for FF of peaches.

The baseline correction using the asymmetric least squares method [27] and normalization to the integral value of the internal standard (trimethylsilyl group of sodium

Fig. 5. (a) Regression vectors and (b) variable importance in projection (VIP) of the partial least squares (PLS) regression models for measuring flesh firmness (FF), water-soluble pectin (WSP), and elapsed days (ED) from the harvest.
2,2-dimethyl-2-silapentane-5-sulfonate; 0 ppm) were applied to the measured NMR spectra, and then the residual water signal region (4.7–4.9 ppm) was replaced to zero. After binning at intervals of 0.02 ppm, the correlation coefficient between the NMR signal and the NIR signal was calculated for all combinations.

The result is illustrated in Fig. 6. The upper panel shows the NIR spectra. The Savitzky-Golay smoothing and the baseline correction were applied to the spectra. The right panel shows the \(^1\)H NMR spectra including sugar, organic acid, and amino acid signals. The center square panel shows the heat map of the correlation coefficient and the cells with high positive/negative values are colored with red and blue, respectively. There are three marked NMR signals that are correlated to the NIR signals in the range of the water absorption band (930–1000 nm), namely anomeric proton of GalUA at 5.27 ppm, methanol at 3.3 ppm, and succinate at 2.38 ppm.

Fruit softening during ripening is due to cell wall changes, including degradation of polygalacturonate chains and solubilization of pectin [34]. Ripening-related increases in polygalacturonase and pectin methyl esterase activity are the most important factor for cell wall modification and softening in peach fruit (Fig. S-3) [28, 35]. In addition to WSP, both GalUA and methanol (see also Fig. S-5) showed a strong negative correlation with the NIR water absorption band. These results suggest that fruit softening associated with cell wall modification changes the state of water in peach fruit. The signal intensities of GalUA in the NMR spectra show a very loose correlation to the WSP quantity determined by m-phenylphenol colorimetric method (Fig. S-4), because GalUA is one of the structural units of WSPs. The consumption of water molecules during hydrolysis (Fig. S-3) may be one of the reasons for a
decrease in the water band in the NIR range (Fig. 3 (b)) [36]. Furthermore, the succinate signal gradually decreases during the ripening in a manner similar to that of chlorophyll content (Fig. S-5). It is well-known that organic acids such as succinate decrease during the ripening of peach fruit [34]. Thus, the results of SHY led us to propose the NIR water absorption band as a possible indicator for peach fruit softening during ripening.

Consequently, the NIR absorptions in the range of 950—1000 nm reflect the amounts of components which monotonically increase or decrease depending on the progress of ripening. SHY analysis never gives us a spectral assignment, however, there is no doubt that the selected components directly and indirectly impact the structure of the prediction model of FF of peaches.

4. Conclusions

Changes in the maturity indices of the melting type peach were investigated during the postharvest period. FF and the WSP content were appropriate for evaluating the degree of maturity because these indices gradually changed in one direction. The prospective results for non-destructive estimations of both the indices were obtained by applying PLS regression to the intact peach spectra in the 500—1000 nm wavelength range. The developed models for measuring FF, WSP, and ED were based on the same information. NIR-NMR SHY analysis revealed that the components which change depending on the progress of ripening such as GalUA, methanol, and succinate contributed to build the prediction models. The variation of the amount of the pigments (at 500—560 and 630—690 nm, especially chlorophyll) is also used to build these models. Moreover, scattering profiles of the fruit tissue seemed to be valuable in improving model accuracy. The proposed non-destructive technique for estimating the FF or the WSP content can be used for standardizing the within-lot maturity in the product distribution and for providing an estimation of the time for fruit consumption by the consumers. Further studies with a large dataset obtained using a variety of cultivars with the aid of quantum chemical calculation are required to verify the reliability of this technique.

Declarations

Author contribution statement

Yasuhiro Uwadaira: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yasuyo Sekiyama: Performed the experiments.

Akifumi Ikehata: Conceived and designed the experiments.
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Competing interest statement

The authors declare no conflict of interest.

Additional information

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