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자외선 유도 형광의 사과 성숙도 평가 적용

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UV/blue Light-induced Fluorescence for Assessing Apple Quality

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Abstract

Chlorophyll fluorescence has been researched for assessing fruit post-harvest quality and condition. The objective of this preliminary research was to investigate the potential of fluorescence spectroscopy for measuring apple fruit quality. Ultraviolet (UV) and blue light was used as an excitation source for inducing fluorescence in apples. Fluorescence spectra were measured from 'Golden Delicious' (GD) and 'Red Delicious' (RD) apples using a visible/near-infrared spectrometer after one, three, and five minutes of continuous UV/blue light illumination. Standard destructive tests were performed to measure fruit firmness, skin and flesh color, soluble solids and acid content from the apples. Calibration models for each of the three illumination time periods were developed to predict fruit quality indexes. The results showed that fluorescence emission decreased steadily during the first three minutes of UV/blue light illumination and was stable within five minutes. The differences were minimal in the model prediction results based on fluorescence data at one, three or five minutes of illumination. Overall, better predictions were obtained for apple skin chroma and hue and flesh hue with values for the correlation coefficient of validation between 0.80 and 0.90 for both GD and RD. Relatively poor predictions were obtained for fruit firmness, soluble solids content, titrational acid, and flesh chroma. This research has demonstrated that fluorescence spectroscopy is potentially useful for assessing selected quality attributes of apple fruit and further research is needed to improve fluorescence measurements so that better predictions of fruit quality can be achieved.

Keywords : Fluorescence, Near-infrared, Ultraviolet light, Apple quality, Fruit maturity.

1. INTRODUCTION

The maturity of apples is a key factor in determining harvest time and their postharvest quality and end uses. Accurate determination of apple maturity is challenging since individual fruit vary greatly on the same tree or between the trees of the same orchard. The current maturity determination practice requires testing a few apple fruit from the orchard by using multiple destructive methods. The quality parameters measured include skin and flesh color, fruit firmness, sugar (or soluble solids), starch, acid, and ethylene production. In addition to being destructive, these measurements are time consuming, inefficient, and prone to operational error. Nondestructive sensing would offer great advantages in maximizing fruit postharvest quality and solving the inconsistent fruit quality problem that is still commonplace in the market.

Near-infrared spectroscopy (NIRS) has been researched to measure fruit internal quality such as sugar or soluble solids content (SSC) (Lammertyn et al., 1998; Lu and Ariana,

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2002). Commercial NIRS systems are recently available for sorting and grading fresh fruit for SSC. NIRS was also used for measuring fruit firmness and other internal quality attributes such as acid; however, the results are much less desirable. Recently, a new technique of using hyperspectral or multispectral scattering, (Lu and Peng, 2004; Polder, 2002; Noh et al., 2007; Noh and Lu, 2009) was proposed for measuring fruit firmness, which showed superior results compared to the NIRS technique.

Fluorescence is a technique for measuring the light of longer wavelengths released from a fluorescing object after it absorbed short-wavelength light. Most plant materials are fluorescing, which is related to their physiochemical activities. When a fruit is illuminated with the light of short wavelengths (e.g., UV), the chlorophyll fluorescence of fruit rises quickly to maximum within a few seconds. Over the next few minutes, the fluorescence quenching occurs, which refers to the phenomena that the photochemical and non-photochemical processes are activated, and chlorophyll fluorescence falls quickly. Eventually a steady state is reached.

Fluorescence measurements are non-destructive, fast, and less expensive in instrumentation. Recently the fluorescence technique was used to investigate biological materials (Kim et al., 2003; Pfefer et al., 2002), detect environmental, chemical, and biological stresses in plants (Gavel and Marsalek, 2004; Kim et al., 2001) and monitor food quality and safety (Kong et al., 2004; Vargas et al., 2004). Chlorophyll fluorescence has been researched for measuring fruit postharvest quality and condition such as defects and ripeness (Bodria et al., 2002; Mir et al., 2001). Song et al. (1997) studied chlorophyll fluorescence for apple fruit during the harvest season and showed that fluorescence may have some practical use in sorting apples or other chlorophyll-containing fruits or vegetables on commercial packing lines. Bodria et al. (2004) used a fluorescence imaging system equipped with a UV-blue actinic light to obtain fluorescence images of fruit in which the gray level of pixels with the firmness of fresh apples. They reported that the R/IR index, defined as the ratio of the signal measured in red and near-infrared bands, was correlated with the chlorophyll content of the fruits, regardless of fruit species and anthocyanin presence, and this index could be used to track the postharvest ripening process for fresh peaches harvested at different maturity stages. Ariana et al. (2003) used multispectral imaging system combined with neural network analysis to detect apple disorder. They used three different imaging modes (reflectance, visible light induced fluorescence, and UV induced fluorescence) to classify various types of apple disorder.

However, published research (Noh and Lu, 2007) also indicates that chlorophyll fluorescence alone may not be sufficient for measuring ripeness-related quality attributes. Bron et al. (2004) concluded that chlorophyll fluorescence cannot reliably determine the overall quality of papaya fruit, and it should be used in complementing other quality measurement techniques.

This research was aimed at developing a fluorescence spectroscopic technique for measuring multiple apple quality parameters including fruit firmness, SSC, acid, and skin/flesh color. This paper reports on results from the preliminary study of using fluorescence spectroscopy to measure postharvest quality parameters of apple fruit.

2. MATERIALS AND METHODS

A. Apple Samples

The varieties of apples used in this research were Golden Delicious (300) apples and Red Delicious (350) apples. Those apples were harvested from the orchard of Michigan State University Clarksville Horticultural Experiment Station (Clarksville, MI) and a commercial orchard in Michigan. The apples were stored in controlled atmosphere (CA) for five months prior to the experiment. During the experiment the apples were refrigerated in a walk-in cooler at 5℃. The apples were kept at room temperature for at least 15 hours before fluorescence measurements and standard destructive quality measurements were carried out.

B. Fluorescence Spectroscopic System

A UV/blue light induced fluorescence spectroscopic system was assembled, which is shown in Figure 1. An ozone free broad band xenon lamp (Spectra-Physics, Stratford, CT, USA) was used as a continuous excitation source for UV light induced fluorescence. Two filters were used to obtain UV light from the broad band xenon light; they were a band pass filter centered at 400 nm with a bandwidth of 70 nm and a short pass filter at the cutoff wavelength of 450 nm. After filtering, the broad band light was changed to the UV/blue light with the cutoff wavelength at 450 nm. The filtered UV/blue light was delivered to the fruit through a

liquid light guide, which had high transmittance in the UV and blue light region.

A single fiber optic spectrometer (Model S2000, Ocean Optic, Dunedin, FL, USA) with a reflectance probe (the numerical aperture of 0.22) was used for acquiring fluorescence spectra from apple fruit. The spectroscopic system had an effective spectral region from 500 nm to 1100 nm with a 1.25 nm spectral resolution.

The incident UV/blue light beam was 30 mm in diameter. The detecting probe was positioned 57 mm above from the apple to cover a 15.3 mm diameter area (Fig. 1).

Each apple was placed into the holder with the stem-calyx end horizontal. Fluorescence spectra were taken from the equatorial area of each apple and saved in the computer. The fluorescence spectroscopic system captured the first fluorescence spectrum from the apple one minute after the fruit was illuminated with UV/blue light. Fluorescence spectra were acquired again from the same apple after three and five minutes of illumination, at which time fluorescence has stabilized. Due to the color difference of apples (Golden delicious is green and Red Delicious is red), the integration time was set at 1000 ms for Golden Delicious apples and 1500 ms for Red Delicious apples. Fluorescence was not measured at the beginning of excitation because the light shutter was manually operated (manual operation time was greater than integration time of the system) and could not control the illumination time accurately. The longer integration times used for the spectrometer also made it impossible to accurately measure fluorescence during the initial few seconds.

C. Apple Quality Measurements

1) Color measurement

After fluorescence measurements, skin color of the apples was measured from the same area by using a colorimeter (Model CR-400, Konica Minolta Sensing Inc., Osaka, Japan) with an 8 mm circular measurement area. Flesh color measurements were performed on the same area after the skin of the fruit was removed. The color was recorded in the format of CIE XYZ color space (also known as CIE 1931 color space). It was then converted into Lab color space (CIE L*a*b*) which is an absolute color space. Chroma ($\sqrt{a^{*2}+b^{*2}}$) and hue ($\arctan(b^*/a^*)$) was derived from Lab color space.

2) Firmness measurement

A texture analyzer (Model TA. XT2i, Stable Micro Systems, Surrey, UK) with an 11 mm Magness-Taylor (MT) probe was used for measuring the firmness of the apples. The MT firmness test was performed on the same area for the flesh color measurement. The probe was penetrated into the apple for a depth of 9.0 mm at a loading rate of 2.0 mm/s. Force versus distance curves were recorded and maximum forces were used as a reference measure of apple firmness.

3) Soluble solids content measurement

Soluble solids content expressed in Brix was measured from the juice released during the MT test with a hand-held digital refractometer (Model PR-101, ATAGO CO, Tokyo, Japan).

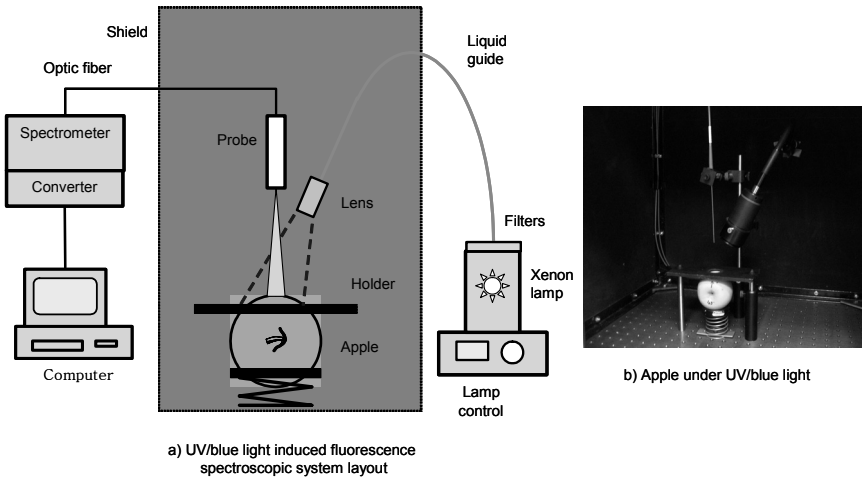

Fig. 1 A UV and blue light induced fluorescence spectroscopic system.

4) Acid content measurement

Acid content expressed in titrational acid or T-acid (%) was measured by a titration method with 0.5% malic acid as a reference. To facilitate juice extraction, the apples were first frozen in a freezer after the above mentioned tests had been completed, and they were then thawed at room temperature to be juiced. Six ml of apple juice was titrated using 0.1 sodium hydroxide (NaOH) until the equivalent point has been reached (the indicator turned color). The amount of the titrant used was recorded and converted into T-acid in terms of the malic acid equivalent.

D. Partial Least Squares (PLS) Algorithm

Samples were divided into two groups; one is calibration and the other is validation. The apples were arranged in ascending order for their MT firmness. The first three apples were selected for calibration and the fourth apple for validation. This procedure was repeated for the rest apples, which yielded 3/4 of the apples for calibration and 1/4 for validation.

Fig. 2 is a flowchart diagram showing the procedure of developing a prediction model. The fluorescence spectra were converted from non-evenly spaced file to an evenly spaced trace (XY 2 Even) and then smoothed using the Savisky-Golay method. Partial least squares was performed on the calibration data. PLS factors were then used to develop a calibration model for predicting apple fruit firmness or other quality parameters.

The initial calibration model development started with 25 PLS factors for 500-1100 nm. Cross validation was performed by calculating the predicted residual error sum of squares (PRESS) for different numbers of factors. Once the number of factors corresponding to the minimum PRESS was determined, the spectral and concentration residuals were calculated and plotted to detect outliers using a Mahalanobis threshold of 1.8. After these outliers were removed, a new calibration procedure was started. The PRESS plot was again used in the same way to determine the number of factors. The calibration model with the number of factors chosen in the 2nd iteration was saved. This calibration model then ran predictions for the validation data set.

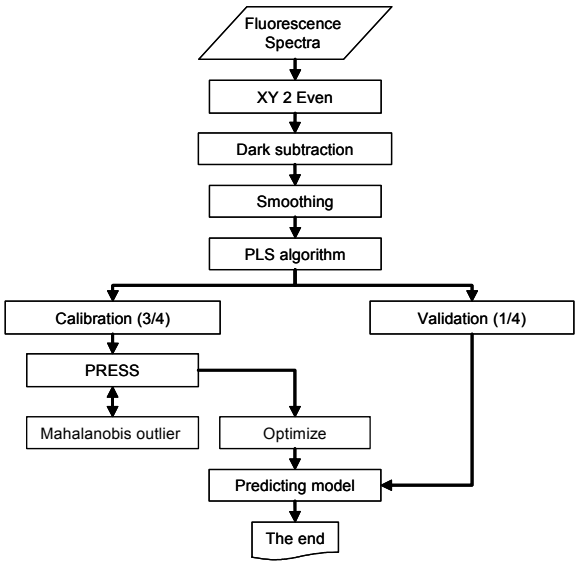

Fig. 2 Flowchart diagram of the calibration model development.

The same procedure was followed for developing SSC, T-acid, and skin/flesh color prediction models.

3. RESULTS AND DISCUSSION

The statistics of fruit firmness, SSC, T-acid, and skin and flesh color for the tested apples are summarized in Table 1. The firmness distribution of the tested apples was relatively

Table 1 Statistics of destructive quality measurements of 300 Golden Delicious (GD) apples and 350 Red Delicious (RD) apples

Variety	Statistic	Firmness [N]	SSC [%]	T-acid [%]	Skin chroma	Skin hue	Flesh chroma	Flesh hue
GD (300)	Max	77.2	17.8	0.63	57.4	1.57	33.7	-1.19
	Min	27.3	9.1	0.13	37.0	-1.55	17.3	-1.46
	Mean	50.7	12.9	0.37	49.1	-1.03	25.6	-1.35
	Std	14.1	1.5	0.19	3.9	0.87	2.8	0.05
RD (350)	Max	97.3	18.7	0.41	44.1	1.31	34.4	-1.20
	Min	21.2	11.2	0.11	20.8	0.19	16.2	-1.55
	Mean	56.1	13.7	0.23	32.9	0.44	24.9	-1.33
	Std	13.9	1.3	0.06	4.5	0.14	2.9	0.06

uniform in the range from 30 N to 70 N for both varieties.

Fig. 3 shows fluorescence spectra GD apple and RD apple for three different time periods of light excitation. The two apples had different intensities of fluorescence due to the varietal difference and different integration times used. The GD apple had a relatively large peak at 536 nm compared to the RD apple. Both apples had the maximum peak at 685 nm and a shoulder around 740 nm.

The fluorescence shoulder at 740 nm was due to energy transfer from Photosystem II to Photosystem I. The peak fluorescence at 685 nm was to the chlorophyll a of Photosystem II units(Kitajima and Butler, 1975).

The fluorescence of apples decreased with time. This phenomenon is termed fluorescence induction kinetic, fluorescence transients, or Kautsky effect (when the excitation light is switched on, fluorescence rises quickly and then drops slowly until it reaches a steady state after a few minutes due to the change of photochemical quenching). The fluorescence change was much greater during the first minute than for the later time periods. After three minutes, fluorescence changed little and it reached the steady state in five minutes. As shown in Fig. 3, the fluorescence profile in three minutes is not distinguishable from the one in five minutes. Due to the instrumentation limitation, fluorescence was not captured for the test apples at the beginning of light illumination.

Values for the correlation (R) the standard error of calibration (SEC) and validation (SEV) the prediction model at one, three, and five minutes are shown in Table 2 and 3 for each variety. And MT Firmness, SSC, and T-acid prediction results (validation) of GD and RD apples for the five-min fluorescence model are shown in Fig. 4.

A. Golden Delicious Apples

The prediction models for the one, three and five minute fluorescence data produced similar calibration results with

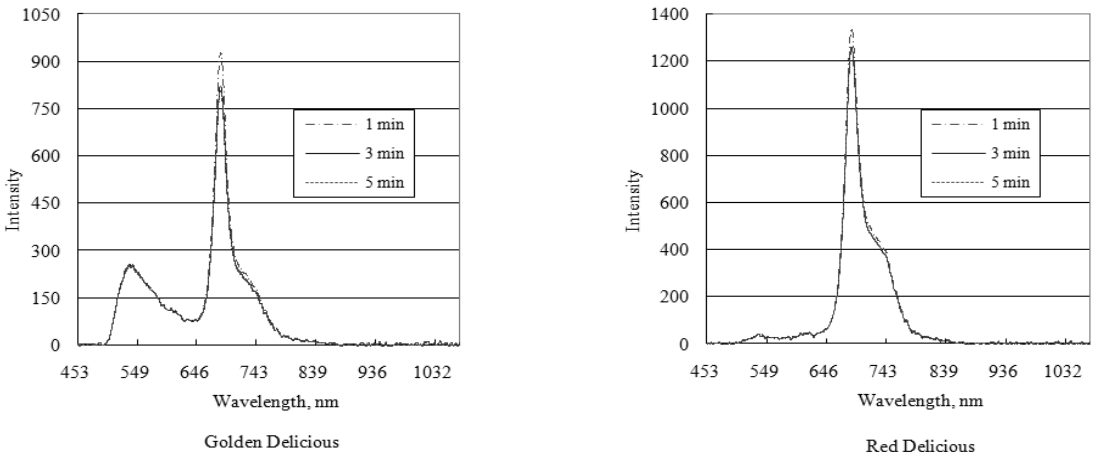

Fig. 3 Fluorescence spectra of two apples at different times after continuous UV/blue light illumination.

Table 2 Calibration and validation results as measured by the correlation coefficient R and the standard error for calibration (SEC) and validation (SEV) for the firmness, SSC, T-acid and skin/flesh color of 300 Golden Delicious (GD) apples

	1 min				3 min				5 min			
	Calibration		Validation		Calibration		Validation		Calibration		Validation	
GD	R	SEC	R	SEV	R	SEC	R	SEV	R	SEC	R	SEV
Firmness	0.91	5.9	0.70	10.5	0.72	9.8	0.70	10.2	0.81	8.2	0.76	9.3
SSC	0.85	0.8	0.56	1.3	0.85	0.8	0.59	1.2	0.72	1.0	0.57	1.2
T-acid	0.84	0.06	0.66	0.08	0.72	0.07	0.66	0.08	0.84	0.06	0.56	0.09
Skin chroma	0.85	2.06	0.85	2.09	0.85	2.04	0.84	2.17	0.86	2.02	0.85	2.09
Skin hue	0.84	0.47	0.82	0.52	0.84	0.47	0.81	0.53	0.83	0.48	0.80	0.54
Flesh chroma	0.70	2.01	0.57	2.36	0.65	2.12	0.54	2.39	0.70	2.00	0.56	2.41
Flesh hue	0.83	0.03	0.83	0.03	0.83	0.03	0.84	0.03	0.83	0.03	0.84	0.03

Table 3 Calibration and validation results as measured by the correlation coefficient R and standard error for calibration (SEC) and validation (SEV) for the firmness, SSC, T-acid and Skin/Flesh color of 350 Red Delicious (RD) apples

	1 min				3 min				5 min			
	Calibration		Validation		Calibration		Validation		Calibration		Validation	
RD	R	SEC	R	SEV	R	SEC	R	SEV	R	SEC	R	SEV
Firmness	0.90	6.1	0.63	10.8	0.86	7.2	0.63	10.9	0.87	6.8	0.59	11.9
SSC	0.50	1.1	0.54	1.1	0.49	1.1	0.53	1.1	0.49	1.1	0.53	1.1
T-acid	0.57	0.05	0.62	0.05	0.58	0.05	0.63	0.05	0.58	0.05	0.63	0.05
Skin chroma	0.91	1.85	0.83	2.49	0.88	2.15	0.80	2.73	0.85	2.37	0.80	2.70
Skin hue	0.86	0.08	0.90	0.06	0.84	0.08	0.88	0.07	0.84	0.08	0.88	0.07
Flesh chroma	0.47	2.55	0.36	2.61	0.54	2.42	0.46	2.49	0.54	2.42	0.45	2.50
Flesh hue	0.90	0.03	0.83	0.03	0.85	0.03	0.70	0.04	0.90	0.03	0.79	0.04

the one-min model being marginally better for selected quality indexes than the three- and five-min models. The one-min fluorescence model gave the best firmness predictions as measured by the values for the correlation coefficient and SEC. The three-min fluorescence model had the lowest value for R compared to the one- and five-min prediction models. A similar trend was observed in the model predictions of T-acid and flesh chroma. For skin chroma and hue, the calibration results from the three prediction models were about the same. The one- and three-min prediction models were better than the 5-min fluorescence model in SSC prediction. All three models had poor calibration results for the flesh chroma prediction in terms of R and SEC.

When the prediction models were used to predict validation samples, the results were much worse than calibration results, which are especially evident for MT firmness, SSC, T-acid, and flesh chroma. The five-min firmness prediction model had a higher value for the correlation coefficient (R=0.76) compared to the one- and three-min prediction models. Values for R from the one- and three-min firmness prediction models were the same (R=0.70). The skin/flesh hue and skin chroma prediction models showed more consistent results in calibration and validation, indicating that the models were robust in predicting these quality indexes.

Overall, the prediction models for one, three, and five minute fluorescence data gave similar validation results. This suggests that fluorescence measurements can be carried out in one minute or even in a shorter time period without affecting prediction results. This is important in developing a rapid, nondestructive technique for sensing apple fruit quality.

B. Red Delicious Apples

The firmness prediction results for RD apples were similar to those for GD apples. The one-min firmness prediction model gave the best firmness predictions with values for R and SEC of 0.90 and 6.0 N, respectively. The three- and five-min firmness prediction models had similar values for R and SEC (Table 3).

The prediction models for all three time periods did not give good predictions in SSC, T-acid, and flesh chroma. The prediction models had poor prediction results for SSC and flesh chroma with values for the correlation coefficient lower than 0.55.

For validation, the one- and three-min firmness prediction models gave the same validation results with a value for R of 0.63. Again, poor validation results were obtained for SSC, T-acid, and flesh chroma, and much better validation results for skin chroma and hue and flesh hue.

Although no statistical tests were performed, the results for RD apples again suggest that the prediction models based on one-min fluorescence data were not much different from the ones based on the five-min fluorescence data, which represented the steady-state fluorescence.

The results in Table 2 and 3 showed that the overall calibration and validation results for all quality indexes for the two apple varieties were similar. Fluorescence spectroscopy gave good predictions of skin chroma and hue and flesh hue but had poor predictions SSC, T-acid and flesh chroma. No differences were observed in the prediction results from the fluorescence data collected at one, three, and five minutes of continuous UV/blue illumination. This suggests that fluorescence spectra may be measured after the fruit is illuminated with UV/blue light, which would be

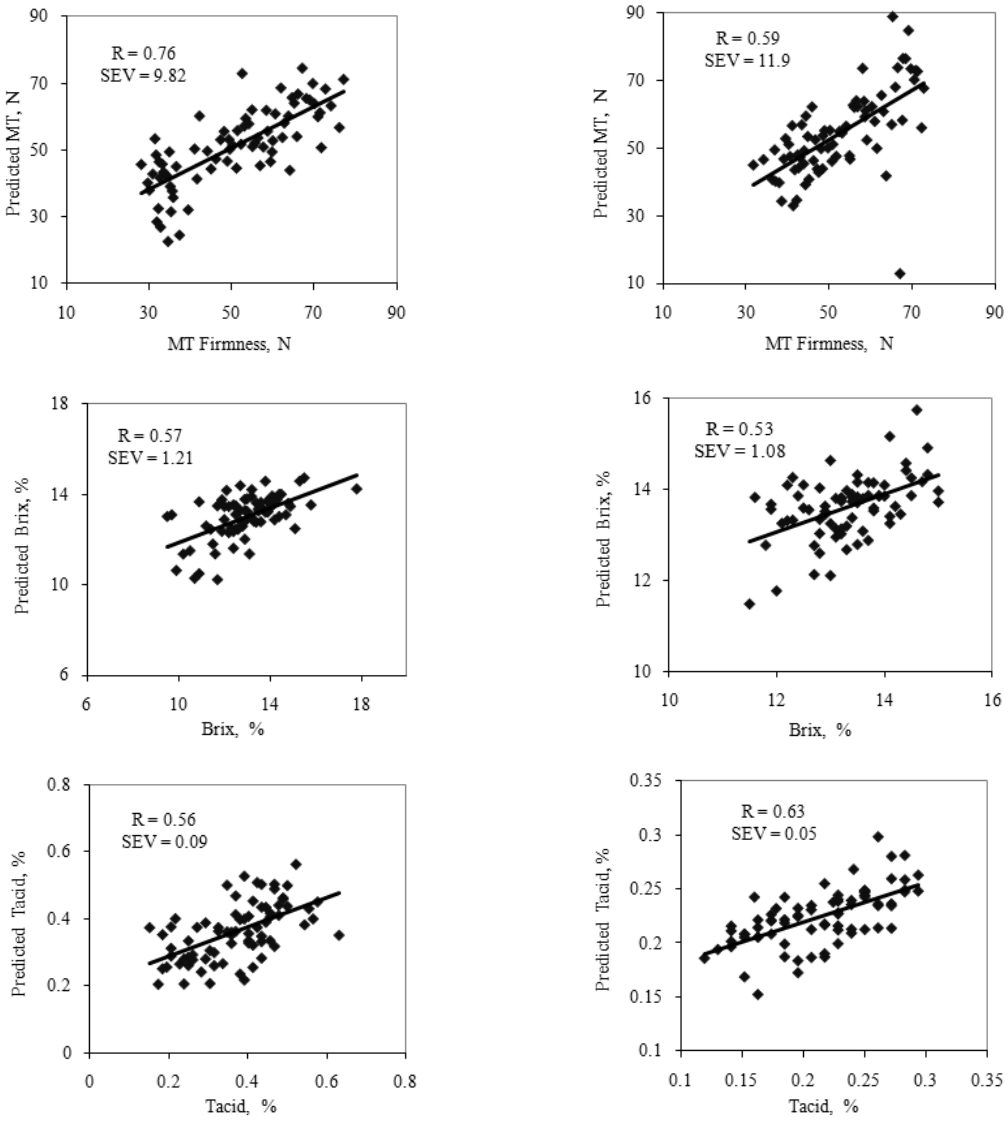

Fig. 4 MT Firmness, SSC, and T-acid prediction results of GD(left) and RD(right) from the 5 min. fluorescence model.

advantageous for rapid measurement of fruit quality. Relatively poor results for T-acid could be in large part attributed to the measurement procedure used in this study. The titration test was performed on the juice extracted from whole apples whereas fluorescence measurements were only taken from one small area on the equatorial section of each fruit. Firmness, SSC and T-acid are known to vary within an apple fruit.

This preliminary research indicates that fluorescence spectroscopy can be potentially useful for assessing quality of apple fruit. However, values for the correlation coefficient for firmness, SSC and T-acid are still low. Hence, further improvements in instrumentation and data analysis are needed before the fluorescence spectroscopy technique becomes a viable tool for quality evaluation of fruits. Fluorescence spectroscopy may need to be integrated with other nondestructive sensing techniques such as near-infrared reflectance spectroscopy in order to achieve superior results.

4. CONCLUSION

Fluorescence spectra for both Golden Delicious (GD) and Red Delicious (RD) apples were collected in the spectral region between 500 nm and 1100 nm when the fruit were illuminated with ultraviolet/blue (UV/blue) light (<450 nm).

The results of this research were summarized as follows

(1) Fluorescence decreased with time during the initial

three minutes of UV/blue light illumination and reached a steady state within five minutes.

(2) The fluorescence prediction models based on the data collected at one, three, and five minutes of continuous UV/blue light illumination had about the same validation results for all quality indexes.

(3) Good predictions were obtained for skin hue and chroma and flesh hue for both GD and RD apples with values for the correlation coefficient (R) of validation between 0.80 and 0.90. And poor predictions were obtained for soluble solids content, titrational acid content and flesh chroma.

(4) Fluorescence was also related to fruit firmness, but the correlation was relatively low; the best R value for validation was 0.76 for GD and 0.63 for RD.

This preliminary research demonstrated that fluorescence spectroscopy can be potentially useful for assessing quality of apple fruit. However, improvements in fluorescence measurements are needed to achieve better predictions of apple quality indexes.

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