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# Application of Time-of-Flight Near Infrared Spectroscopy for Detecting Sugar and Acid Contents in Apples

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A newly constructed optical measurement system was introduced to nondestructively measure the composition of the inside of an apple by time-of-flight near-infrared spectroscopy (TOF−NIRS). As sugar content increased, optical parameters concerned with time-resolved profile of transmitted pulsed light (the attenuance of peak maxima, $At$, the time delay of peak maxima, $\Delta t$, and the variation of full width at half-maximum, $\Delta w$) decreased gradually. When the acid content increased, $At$ and $\Delta w$ increased; however, a significant tendency could not be found for $\Delta t$. $At$, $\Delta t$, and $\Delta w$ were employed as the explanatory variables for multiple linear regression, principle component regression, and partial least-squares analysis. It was possible to predict both sugar and acid contents in an apple with high precision by TOF−NIRS. Especially, the superiority of TOF−NIRS lied in more precise determination of acid content.

**KEYWORDS: Time-of-flight; near-infrared spectroscopy; *Malus sylvestris* var. *domestica* "Fuji"; soluble solids; free acids; time-resolved profile; chemometrics**

## INTRODUCTION

Consumer demands with respect to agricultural products are becoming increasingly diverse. The producer must not only supply them safely while maintaining freshness but also needs to ensure the taste and nutritional values. Therefore, a nondestructive measurement system that can accurately monitor these indices simultaneously with minimum time and effort is very desirable for marketing of agricultural commodities. In the past few decades, many researchers have focused on the potential use of near-infrared (NIR) spectroscopy, a practical spectroscopic procedure for detection of organic compounds in matter. In the fields of agriculture, food, medicine, paper, polymer, etc., intense and aggressive interest has been directed toward NIR spectroscopy because of its nondestructiveness, accuracy, rapid response, and easy operation (*1*, *2*, *3*).

Detection of NIR light from a sample is by either transmittance or reflectance. The transmittance method is very desirable for detecting internal information of matter with large volume, whereas the optical information from diffusely reflected spectra is confined to the subsurface layer of samples. It is especially important that progress be made in developing an NIR transmission device for detection of internal characteristics of high moisture fruit and vegetable products (*4*). However, behavior of transmitted light from an agricultural product is directly affected by both physical and chemical properties of the tissues (*5*), making it very difficult to examine in detail the optical characteristics of the tissue and the output origin of transmitted light for the accurate evaluation of the sample constituents.

To resolve such problems, some researchers have given attention to time-of-flight (TOF) spectroscopy, which uses short pulses of illumination where a portion of the light is scattered, but most of the light propagates through the sample. A time-resolved measurement, or time domain system, could provide the TOF information of the detected light. Patterson et al. (*6*) theoretically proposed the usefulness of the time-resolved reflectance and transmittance for the noninvasive measurement of tissue optical properties. Profio (*7*) or Sevic et al. (*8*) reported on the properties of light scattering in various samples. Leonardi and Burns (*9*, *10*) investigated quantitative measurements in scattering media on the basis of TOF spectroscopy with analytical descriptors. They revealed that experimental analysis of the time-resolved profile was very efficient for estimating the absorption and scattering coefficient.

Recently, Tsuchikawa and Tsutsumi (*11*) have proposed a hyphenated technique between TOF and NIR spectroscopy that is named as TOF-NIRS. This system combined the best features of the spectrophotometer (i.e., tunability of wavelength) and the laser beam (i.e.; permeability of incident light), and more advantageously, the behavior of transmitted light could be observed by time-resolved state within very short time domain. The system, made up of a parametric tunable laser and a NIR photoelectric multiplier, was constructed on the basis of such new concept, where a time-resolved profile of transmitted output power was measured with nanosecond sensitivity. In the previous report (*12*), we investigated in detail the variation of time-resolved profiles resulting from water-core tissue in apple, laser beam wavelength, and detection position of transmitted light. Light scattering state and substantial optical path length in apple were also estimated, which were key factors for the

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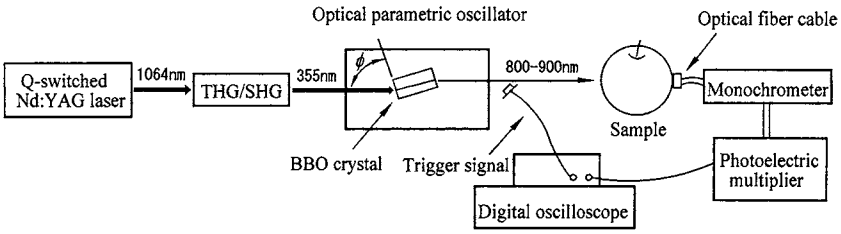


**Figure 1.** Schematic diagram of the measuring system (TOF-NIRS).

correct optical measurements. These parameters were also strongly dependent on detection position and wavelength of the laser beam. The substantial optical path length calculated from the time delay of the peak maxima in time-resolved profile $\Delta t$ at $\lambda = 800$ nm was 10 to 17 times, while that for $\lambda = 900$ nm varied from 6 to 11 times the distance of the diameter of the fruit. Results indicated that the optical parameter for detection of water core was $\Delta t$. It was therefore revealed that the intensity of output signal (e.g., absorbance) was not necessarily the best parameter for nondestructive determination.

The variation of time-resolved profile with sugar and acid contents in Satsuma mandarin was furthermore investigated (*13*). A series of results suggested that time-resolved profile was governed not only by the concentration of soluble solids or free acids but also by other inherent factors of a fruit, for example, an optical characteristic of tissue or difference in refractive index between the tissue substance and the fruit juice. Such results should be applicable for the other fruits. Also, in this case, the usefulness of optical parameter in time-resolved profile as nondestructive measurement was revealed.

On the basis of these suggestful results, this research aimed to clarify the applicability of TOF-NIRS to the detection of sugar and acid contents in apple. First, the effect of these constituents and the laser beam wavelength on the time-resolved profile was fully examined to estimate the optical parameter preferable for detecting internal quality. Second, it was aimed to find not only wavelength-dependent characteristics but also time-dependent characteristics of experimental data by using a simple processing method. Normally, the chemometrics by NIR spectra employs the absorbance as the explanatory variables, where only wavelength-dependent characteristics of the materials could be considered. In this case, it is very difficult to precisely evaluate the small amount of constituent such as acid content in a fruit. On the other hand, the chemometrics by TOF-NIRS would be related to both wavelength- and time-dependent characteristics parameter as the explanatory variables, where the light-absorption condition and light-scattering phenomena in a sample are included. Therefore, it may be possible to detect the acid content in a fruit on the basis of such a new optical concept. This report is a first attempt to introduce such novel statistical approach to horticultural products. The performance of several chemometric approaches (e.g., multiple linear regression (MLR), principle component regression (PCR), and partial least-squares (PLS) analysis) with TOF-NIRS were compared to them with the normal NIR spectroscopy using reflectance technique.

In the field of medical science, such time-domain method is expected from developing optical tomography techniques, which can especially be used for the noninvasive detection of breast cancer (*14*) or hemoglobin concentration changes associated with the neural activation of the human brain (*15*). Therefore, TOF-NIRS should be also applicable for image construction concerning the agricultural products. These basic data can be helpful in realizing an in-process measurement system for the fruit industry.

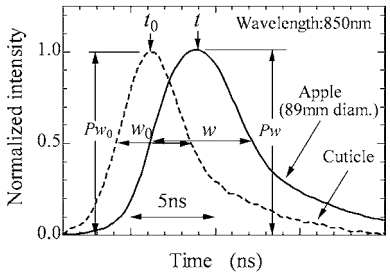


**Figure 2.** Normalized time-resolved profiles of an apple and its cuticle.

## MATERIALS AND METHODS

**Measuring Apparatus.** A schematic diagram of the measuring system used is illustrated in **Figure 1**. The system consisted of an exciter laser (Surelite I-10, Continuum Electrooptics Inc., Santa Clara, Calif.), an optical parametric oscillator (X2852, HAMAMATSU Photonics Co., Hamamatsu, Japan), a monochromator (SG-250 KOKEN KOGYO Co., Tokyo, Japan), a near-infrared photoelectric multiplier (R5509−71, HAMAMATSU Photonics Co., Hamamatsu, Japan), and a digital oscilloscope (Model 9362, LeCroy Co., Chestnut Ridge, N. Y.). A Q-switched Nd:YAG laser having an output energy of 60 mJ/pulse at 335 nm, a pulse width of 5 ns, a pulse repetition frequency of 10 Hz, and a beam diameter of 6 mm was employed as the exciter laser. The wavelength of the pulsed laser ($\lambda$) was tuned from 800 to 900 nm at a stepwidth of 10 nm by the optical parametric oscillation of a BBO ($\beta$-$BaB_2O_4$) crystal (*16*). The output power of the tuned laser beam ranged from 8 mJ/pulse (at 800 nm) to 5 mJ/pulse (at 900 nm). However, in this power range, the spectral line width of the laser beam varied with the wavelength from 1 to 2 nm. The monochromator was utilized to keep the spectral line width constant at 0.1 nm.

The transmitted output power from the sample was measured by an NIR photoelectric multiplier having a spectral response ranging from 300 to 1700 nm, which was cooled to −80 °C, through an optical fiber cable having a diameter of 7 mm. A Si pin-type photodiode was placed near the optical parametric oscillator to generate a trigger signal. The optical fiber cable was directly in contact with the apple, which was enclosed in aluminum foil to keep out stray light. The sample, the cooling box containing the NIR photoelectric multiplier, and the optical fiber cable were also covered with black cloth. The sampling time and the number of times the transmitted output power was averaged were 100 ns and 300 times, respectively.

**Outline of Time-Resolved Profile.** Time-resolved profile refers to the variation in intensity of the detected light beam with time. The combined effects on the time-resolved profiles of the sugar and acid contents and the laser beam wavelength ($\lambda$) were investigated in detail. We focused on some typical parameters representing the variation of time-resolved profile. The normalized time-resolved profiles of an apple (89 mm in diameter) and its cuticle (1.0 mm in thickness) are illustrated in **Figure 2**. When the NIR photoelectric multiplier was used, the linearity of the input signal with respect to the photo sensitivity had to be considered. As the intensity of input pulse was very large compared to that of the transmitted output power from the sample, there may have been a stable relation between the two values. Therefore, the time-resolved profile of the cuticle was employed as the reference.

Variations in peak maxima, the time delay of peak maxima, and the full width at half-maximum of the profile were examined under the

experimental measuring conditions. The measure of attenuance ($A_t$) was defined as follows:

$$A_t = \log\left(\frac{Pw_0}{Pw}\right) \quad (1)$$

where $Pw_0$ and $Pw$ indicate the peak maxima of the reference and the object, respectively. The measure of time delay of peak maxima ($\Delta t$) was expressed as follows:

$$\Delta t = t - t_0 \quad (2)$$

where $t_0$ and $t$ indicate the time at peak maxima of the reference and the object, respectively. The variation of the full width at half-maximum value of the profile ($\Delta w$) was also expressed as follows:

$$\Delta w = w - w_0 \quad (3)$$

where $w_0$ and $w$ indicate the full width at half-maximum value of the profile for the reference and the object, respectively.

**Normal NIR Measurement.** In this study, the diffusely reflected NIR spectra was measured by using commercial spectrophotometer to compare the usefulness as the explanatory variable in calibration equation with those from TOF-NIRS. The yypical instrument employed was the InfraAlyzer 500 from Bran+Luebbe Co.. It includes a diffraction grating and an integrating sphere for obtaining spectral data. The optical fiber probe was used for direct attachment between the sample and the detector. In this system, NIR spectra with high wavelength resolution (about 0.1−1 nm) can be measured continuously using diffraction gratings. However, the optical penetration length of incident monochromatic beam into the agricultural products (e.g., apple or Satsuma mandarin) should be limited to less than 2 mm. The wavelength of incident light varied from 800 to 900 nm at a stepwidth of 2 nm.

**Fruit Samples.** The 30 samples used were Fuji apples [*Malus sylvestris* (L.) Mill. var. domestica (Borkh. Mansf.)] harvested November 1−5, 2001 from an orchard in Aomori, Japan. The fruit diameter, fruit weight, sugar content, and acid content varied in 80.1−88.4 mm, 249−309 g, 11.1−13.5 Brix%, and 0.19−0.41%, respectively. In visual evaluation, there were no water-cored apples in the population.

In the case of TOF-NIRS measurement, the equator of an apple was irradiated vertically with the pulsed laser, and the transmitted output power was detected at the opposite face on the equator. The normal NIR measurement and the diffusely reflected spectra were collected by attaching the probe on the same irradiation position as with TOF-NIRS measurement. After the series of optical experiments, the apple was squeezed to produce fruit juice for the measurement of the sugar and acid contents, which should be strictly defined as the refractometric dry substance and conductivity, respectively. The concentration of sugar content was measured with a digital refractometer (IPR 201, ATAGO Co., Tokyo, Japan), while the concentration of free acids was measured with a dual conductivity meter (WM-50EG, DKK-TOA Co., Tokyo, Japan). It should be noted that the measured constituents express the average in whole apple; however, many NIR researchers collected them from a piece of sample where the spectrum was taken. The following discussions are therefore taking into account the relationship between optical signal and "average" constituents in whole apple. Such comparison may be in tune with the information that consumers want to know.

Each sample was measured five times within a given detection position and wavelength of the laser beam. During the measurement periods, no significant changes in time-resolved profile were observed. The following results are therefore presented as the average signal of five measurements.

**Chemometric Techniques for Quantitative Analysis.** In this study, three chemometric modeling (i.e., multiple linear regression (MLR), principle component regression (PCR), and partial least-squares (PLS) analysis) were performed to evaluate average sugar and acid content in apple.

As described above, the optical data for apples were collected by normal NIRS and TOF-NIRS technique. In the case of normal NIR, the diffusely reflected second derivative spectra between 800 nm to 900 nm (stepwise: 2 nm) were employed as explanatory variables. On the other hand, $At$, $\Delta t$, and $\Delta w$ by TOF-NIRS between 800 and 900 nm (stepwise: 10 nm) were also employed as explanatory variables, where each parameter was divided by sample diameter $d$ to compensate for the effect of sample size. The maximum number of explanatory variables theoretically amounted to 33.

In the case of raw, spectral files were imported into Microsoft EXCEL 2002 (Microsoft Co.) and Pirouette (v. 3.02; Infometrix Inc.) for data analysis. Quantitative techniques investigated in this work were MLR (EXCEL), PCR (Pirouette), and PLS (Pirouette), where no pretreatment methods were employed. Because studies were aimed at determining the probability that nondestructive measurement of sugar and acid contents may be accomplished using TOF-NIRS, the number of samples was limited to 30. All data were used for calibration and cross-validation.

## RESULTS AND DISCUSSION

**Variations of Time-Resolved Profile with Sugar Contents in Apples.** As a first step in this study, the variations of time-resolved profile with sugar and acid contents in apple were investigated. The time-resolved profiles at $\lambda = 800$ nm with different sugar contents are depicted in **Figure 3a**, where the acid content and sample size were of the same value. It is known from this figure that the transmitted output power increased with increment of sugar content. On the contrary, an acceleration in peak maxima was also observed as the sugar content increased. The NIR beam at $\lambda = 800$ nm is vigorously scattered with little absorption (*11*). This results in less light scattering, so that the light path time through a sample decreases. The results could be phenomenally explained by the light scattering in an apple, which is diminished as the sugar content increases because the refractive index $n$ of fruit juice generally increases as the sugar content increases in it. The $n$ of substantial cellular tissue should be over 1.5. Therefore, the refractive index difference between the fruit juice and the cellular tissue will decrease with the increase of sugar content, so that light scattering condition resulted in diminishing. Of course, the effect of multiple light scattering caused by the difference in refractive index between the intercellular spaces ($n = 1.0$) and substantial cellular tissue must be examined.

**Variations of Time-Resolved Profile with Acid Contents in Apple.** The time-resolved profiles at $\lambda = 800$ nm with different acid contents are depicted in **Figure 3b**, where sugar content and sample size are of the same value. In this case, the transmitted output power decreased with the increase of acid content. A delay in peak maxima was also observed as the acid content increased.

The increase of acid content will contribute on the increment of refraction or scattering in an apple. Such phenomena may arise from the variation of $n$ in cellular tissue; however, it must be furthermore examined with respect to their cause. Therefore, time-resolved profile of apple varied characteristically with soluble solids and free acids.

**Variations of Optical Parameters with Sugar Contents in Apples.** The variations of representative of three optical parameters for time-resolved profiles (i.e., attenuance $A_t$, time delay of peak maxima $\Delta t$, variation of the full width at half-maximum value of the profile $\Delta w$) with sugar content in apple were investigated in detail. Because the time-resolved profile was strongly affected by the sample size (*12*), $A_t$, $\Delta t$, and $\Delta w$ were standardized by dividing the apple diameter $d$ (e.g., the distance between irradiation position and detection position). **Figure 4**, parts **a**, **b**, and **c** show the variation of $A_t$, $\Delta t$, or $\Delta w$ at $\lambda = 800$ nm standardized by dividing $d$ with the sugar content

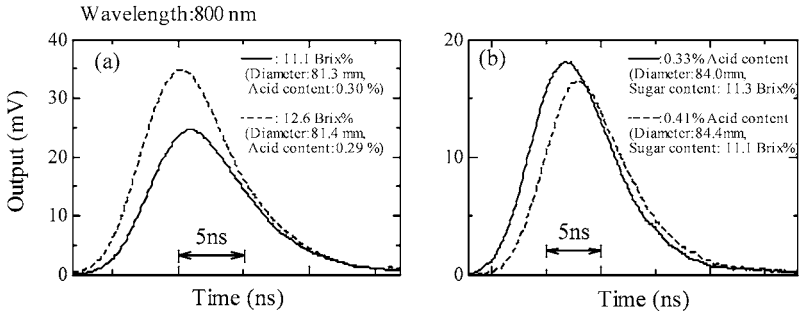


**Figure 3.** Time-resolved profiles at $\lambda = 800$ nm with different sugar or acid contents in apples.

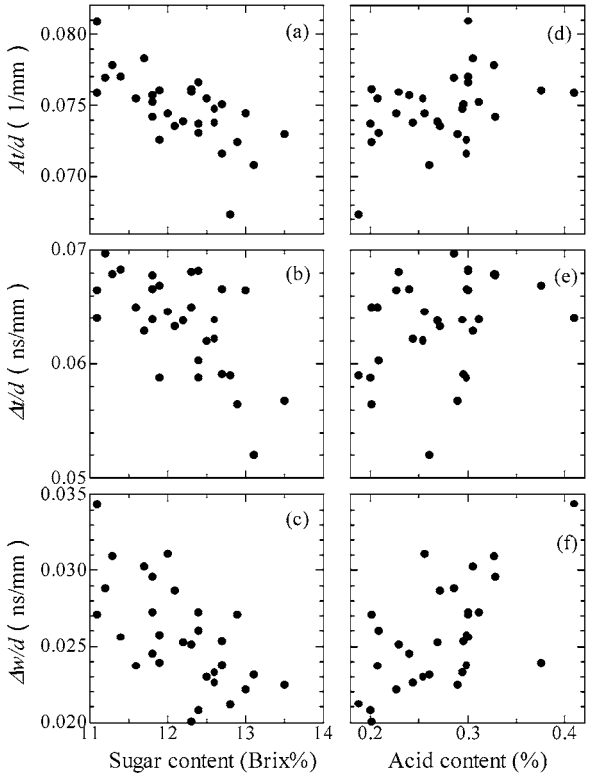


**Figure 4.** Variation of the attenuance of peak maxima $A_t$, the time delay of peak maxima $\Delta t$, and the variation of full width at half-maximum $\Delta w$ at standardized by dividing sample diameter $d$ with the sugar or acid content in apple.

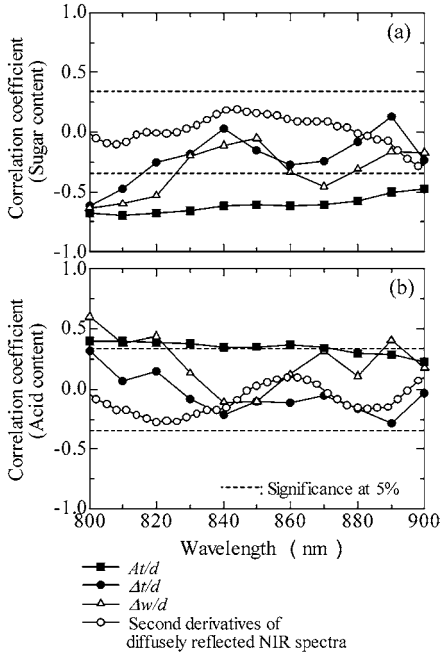


**Figure 5.** Correlation coefficient between sugar or acid contents and $A_t/d$, $\Delta t/d$, or $\Delta w/d$ and second derivatives of diffusely reflected NIR spectra.

in apple. All the parameters ($A_t/d$, $\Delta t/d$, and $\Delta w/d$) decreased as sugar content increased, which could be expressed under the level of significance at 5%. These results conclusively mean that the intensity of transmitted light increased, and the optical path length decreased with decreasing sugar content. These results were observed independently from the pulsed laser wavelength; however, the correlation coefficient between sugar content and optical parameters varied with the wavelength as follows: The correlation coefficients between sugar content and $A_t/d$, $\Delta t/d$, or $\Delta w/d$ are summarized in **Figure 5a**. In this figure, the correlation coefficient between sugar content and second derivatives of absorbance measured by commercial NIR spectrophotometer with reflectance mode is also depicted. $A_t/d$ showed relatively high correlation with sugar content independent of the wavelength. On the other hand, the relationships between sugar content and $\Delta t/d$ or $\Delta w/d$ were statistically significant only at restricted wavelengths. In the case of second derivatives of absorbance, there are none significant within this wavelength range. The effect of acid content should of course be taken into consideration with these analysis.

**Variations of Optical Parameters with Acid Contents in Apples.** The variation of three optical parameters standardized by dividing $d$ with acid content in apple was also investigated. **Figure 4**, parts **d**, **e**, and **f** show the variation of $A_t$, $\Delta t$ or $\Delta w$ at $\lambda = 800$ nm standardized by dividing $d$ with the acid content in apple. Contrary to the obtained results with sugar content, two parameters ($A_t/d$ and $\Delta w/d$) increased as acid content increased, which could be expressed under the level of significance at 5%. However, there was no statistical significance between $\Delta t/d$ and acid content. These results may roughly mean that the intensity of transmitted light decreased, and the optical path length increased as acid content increased. The correlation coefficients between acid content and $A_t/d$, $\Delta t/d$, $\Delta w/d$, and second derivatives of absorbance are summarized in **Figure 5b**. $A_t/d$ showed slightly significant correlation with acid content. On the other hand, the relationships between acid content and $\Delta w/d$ were statistically significant only at short wavelengths. There was no significant correlation between $\Delta t/d$ or second derivatives of absorbance and acid content independent of wavelength.

As the sugar content strongly affects the variation of time-resolved profile, the actual relationship between optical parameters and acid content will not necessarily show low statistical significance. It should be noted that the effect of sugar and acid content with time-resolved profile is inversely related.

**Table 1.** Statistical Parameter for Multiple Linier Regression (MLR) Analysis

| spectroscopic method | constituents | selected wavelength (nm) | $r^a$ | SEC[b] (%) |
|---|---|---|---|---|
| normal NIR | sugar content | 840, 884, 892 | 0.70 | 0.47 |
| | acid content | 816, 846, 900 | 0.57 | 0.046 |
| TOF-NIRS | sugar content | 810[c], 860[d], 800[e] | 0.85 | 0.35 |
| | acid content | 860[c], 890[d], 800[e] | 0.73 | 0.039 |

[a] $r$, regression coefficient between real value and evaluated value. [b] SEC, standard error of calibration. [c] $At/d$. [d] $\Delta t/d$. [e] $\Delta w/d$.

**Table 2.** Statistical Parameter for Principle Component Regression (PCR) and Partial Least Square (PLS) Analysis

| chemometrics | spectroscopic method | constituents | no. of factor | $r^a$ | SEC[b] (%) |
|---|---|---|---|---|---|
| PCR | normal NIR | sugar content | 9 | 0.63 | 0.58 |
| | | acid content | 9 | 0.56 | 0.052 |
| | TOF-NIRS | sugar content | 6 | 0.84 | 0.38 |
| | | acid content | 6 | 0.68 | 0.043 |
| PLS | normal NIR | sugar content | 9 | 0.72 | 0.52 |
| | | acid content | 9 | 0.66 | 0.048 |
| | TOF-NIRS | sugar content | 6 | 0.94 | 0.23 |
| | | acid content | 6 | 0.92 | 0.023 |

[a] $r$, regression coefficient between real value and evaluated value. [b] SEC, standard error of calibration.

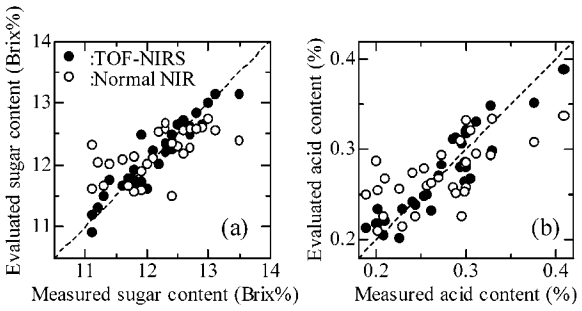


**Figure 6.** PLS analysis for sugar and acid contents in apples.

**Chemometric Approach for the Evaluation of Sugar and Acid Contents Based on TOF-NIRS.** As described above, the time-resolved profile characteristically varied with sugar and acid contents in apples. Then, we attempted to find robust calibration equations for these constituents by MLR, PCR, and PLS analysis, where the optical parameters concerned with the time-resolved profile between 800 and 900 nm were employed as explanatory variables. To compare the accuracy of validation with that of the normal NIR method, the same chemometric techniques based on diffusely reflected spectra in which the second derivative of absorbance between 800 and 900 nm were employed as explanatory variables were also applied. The statistical results are summarized in **Tables 1** and **2**.

**Figure 6** shows the PLS analysis in optimum model for sugar and acid contents in apples. During the cross-validation procedure, one sample was left out, leading to a PLS model with eight or nine principal components. In these figures, black and white circle indicate the value by TOF-NIRS and normal NIR spectroscopy, respectively. In the case of normal NIR analysis, standard error of calibration (SEC) and correlation coefficient between measured and predicted sugar content $r$ were 0.52% and 0.72, respectively. However, in the case of PLS analysis by TOF-NIRS, SEC and $r$ were improved up to 0.023% and 0.94, respectively (see **Table 2**). As mentioned at "fruit samples", the measured sugar and acid contents in this study were average values in an apple, whereas many NIR researchers determined them from a piece of sample. Therefore, the MLR, PCR, and PLS results for normal NIR by this research was worse than those previously reported by other research groups.

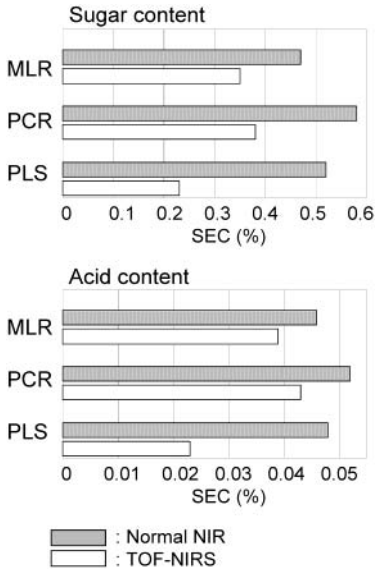


**Figure 7.** SEC results for all multivariate statistical methods.

Interestingly, the usefulness of TOF-NIRS as multivariate statistical methods was clear for the acid content. In the case of PLS analysis by normal NIR, SEC, and $r$ for the prediction of acidity were limited to 0.048% and 0.66, respectively. However, in the case of PLS analysis by TOF-NIRS, SEC and $r$ were improved dramatically up to 0.023% and 0.92, respectively (see **Table 2**). It may be possible to find a good calibration equation concerned with sugar content on the basis of NIR technique by employing a much more explanatory variable, whereas it may be impossible for acid content because of its small amount in an apple. The fit shows that TOF-NIRS is useful to determine the small amount constituent in a fruit.

SEC results for all multivariate statistical methods are summarized in **Figure 7**. The evaluation of sugar or acid content by TOF-NIRS showed a high accuracy compared to the normal method independent of the chemometric procedure used. Upon going from normal NIR to TOF-NIRS, the PLS results have been improved largely; however, the MLR and PCR results were not. The difference of prediction accuracy with chemometric approach should be considered in research to follow. Furthermore, it is also necessary to examine the effect of water content, toughness, or mutuality of an apple on the time-resolved profile for the construction of a robust calibration equation by TOF-NIRS.

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