Near-Infrared Monitoring of the Growth Period of Japanese Pear Fruit Based on Constituent Sugar Concentrations

Munehiro Tanaka and Takayuki Kojima*

Faculty of Agriculture, Saga University, Saga 840, Japan

To develop nondestructive monitoring techniques in plant science, near-infrared spectroscopy (near-IRS) was applied to estimate the growth stages of the fruit of the Japanese pear tree based on the constituent sugar concentrations in the juice. Regular differences were observed among the spectra of juice from pears of different maturity, and the absorption bands of these spectra were assigned to carbohydrate. The multiple linear regression calibration equations of each constituent sugar (namely, sucrose, glucose, fructose, and sorbitol) used three to seven wavelengths as independent variables. The correlation coefficients of each sugar concentration were 0.97, 0.90, 0.99, and 0.98, with standard error of prediction values of 0.17, 0.15, 0.21, and 0.40 respectively. Results of measurement of each constituent sugar concentration in Japanese pear juice by near-IRS indicated that the sugar components changed with the growth period of fruits. Therefore, near-IRS can be used to effectively evaluate the growth stages of the fruit.

Keywords: *Near-infrared spectroscopy; monitoring techniques; Japanese pear; sugar; growth period*

INTRODUCTION

Near-infrared (near-IR) absorption occurs as overtones and combinations of fundamental vibration frequencies in the infrared (IR) region; therefore, absorptivity in the near-IR region is very weak comapred with that in the IR region. However, water absorptivity in the near-IR region is also much weaker than in the IR region, so measurement of samples that have high water concentrations can be done easily, and the absorption based on chemical structure, such as functional groups, can be obtained (Murray and Williams, 1987). For samples such as agricultural products that consist of many constituents, near-IR absorption bands overlap considerably, and the spectral shapes become broad. In these spectra, it is difficult to assign the absorption to chemical structure and the Beer–Lambert law cannot be applied in a straightforward manner. But, with the development of chemmometrics methods, much research and many commercial applications of near-IR spectroscopy (near-IRS) as a means of nondestructive evaluation are materializing (Williams and Norris, 1987).

With regard to measurment techniques in plant science, near-IRS has great advantages because near-IR spectra include both vital structure and general information. This research explores the possibility of applying near-IRS to monitoring techniques in plant science.

The fruit of the Japanese pear tree has a sandy texture and a round shape like an apple. It can be eaten just after harvest because it ripens on the tree and not during storage after harvest (Yamaki et al., 1979a). Therefore, it is very important for a cultivator to know the growth period and accurately estimate the time of fruit harvest based on inner fruit quality. Available estimation indices for the growth period of the fruit from Japanese pear trees are shape, size, peel color, Brix value, change of inner components, and respiration rate for climacteric fruits. Measurements of Brix values or soluble solids for fruits and vegetables by near-IRS were reported by Bellon and Boisde (1989) for fruits, by Dull et al. (1989) for cantaloupe, and by Kawano et al. for peach (1992b) and satsuma mandarin (1992a), etc. These methods are used commercially primarily for ripe crops and not for immature fruit. Measurements of inner components for growing Japanese pear fruit were reported by Yamaki et al. for sugars (Yamaki et al., 1979a), hemicellulose (Yamaki and Kakiuchi, 1979), and cell wall polysaccharides and monosaccharides (Yamaki et al., 1979b), etc., but these were complex chemical analyses and therefore impractical methods for use in the orchard. Climacteric rise was reported by Burg et al. (1962) and Biale (1964) etc., but for applied climacteric rise to estimate a fruit ripening, continuous measurement of respiration is required and therefore this method is also impractical in the orchard.

Endo (1973) reported that the growth phase of Japanese pear fruit could be separated into four stages: the first is the early growth stage, the second is a retarded growth stage, the third is the maximum growth stage, and the fourth is the ripening stage. At the cellular level, the first and second stages are separated on the basis of cellular division, the third stage is a cell-thickening stage, and the fourth stage is a ripening stage. Yamaki et al. (1979a) reported the relationship between the growth phase and change of constituent carbohydrate concentrations. The fruits of Japanese pear contain four constituent sugars; namely, sucrose, glucose, fructose, and sorbitol. As the fruit ripens, the total sugar concentration increases gradually, but changes in each sugar concentration do not occur at the same time. In Japanese pear, photosynthesis products translocate to fruits as sorbitol, and young fruits store sorbitol during the cellular division stage. After a cell-thickening stage, sorbitol is metabolized vigorously to fructose and glucose, which are stored as starch. In the fruit-ripening stage, a climacteric rise occurs, starch decreases, and sucrose increases. Thus, changes of each sugar concentration for Japanese pear fruits during the growing season can indicate the growth phase and the degree of ripeness.

* Author to whom correspondence should be addressed (fax +81-952-28-8768; e-mail kojimat@cc.saga-u.ac.jp).

Near-IR Monitoring of Pear Growth

J. Agric. Food Chem., Vol. 44, No. 8, 1996 **2273**

Table 1. Statistic Values of Calibration and Prediction Sets of Japanese Pear Juice Cultivated in 1993[a]

sugar	caliblation set				prediction set			
	n^b	range	mean	SD[c]	n^b	range	mean	SD[c]
sucrose	98	0–3.00	0.43	0.73	35	0–2.85	0.56	0.85
glucose	98	0.19–1.88	1.10	0.38	35	0.84–1.80	1.28	0.21
fructose	99	0.24–6.47	3.51	1.82	34	2.94–6.30	4.62	0.93
sorbitol	96	2.04–6.87	3.86	1.25	36	2.05–6.84	3.73	1.29

[a] Unit used, % (w/w). [b] n, number of samples. [c] SD, standard deviation.
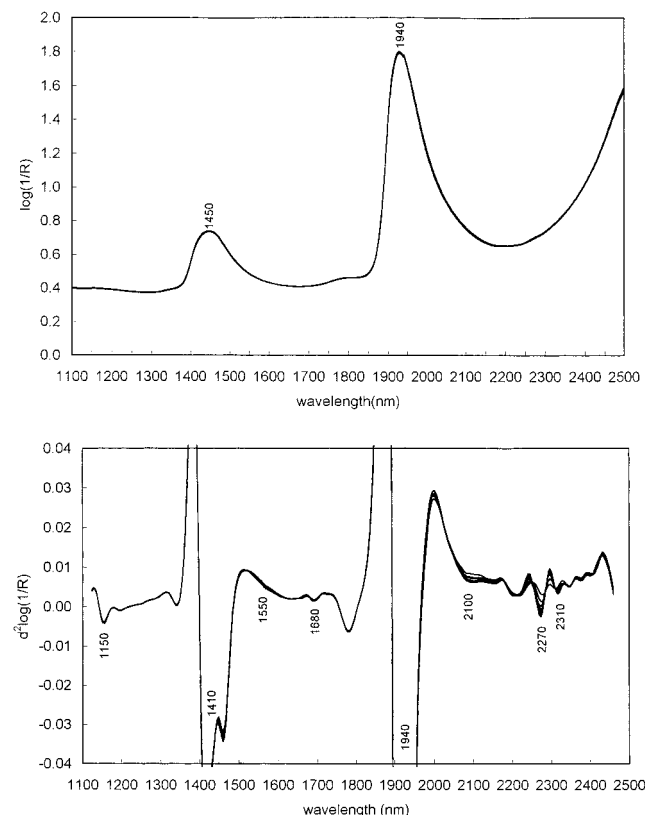
Figure 1. Averaged raw (a, top) and second-derivative (b, bottom) spectra of Japanese pear juice in each harvested day.

In this study, to develop the near-IR monitoring techniques for intact Japanese pear fruits, the constituent sugars in the juice of Japanese pear fruits were used to elucidate the growth period and estimate the ripeness of the fruit. As a quantitative method, near-IRS has good potential as a rapid and easy measure that does not require any onerous pretreatment. Measurements of constituent sugars of agricultural products by near-IRS were reported by Kawano et al. (1991) for sucrose content in sugar cane juice, by Lanza and Li (1984) for sugar content in fruit juices, and by Giangiacoma and Dull (1986) for individual sugars in aqueous mixtures, but these were not for the growing crops.

Multiple linear regression (MLR) was adopted as the quantitative data analysis methods for near-IRS. The calibration equations of each constituent sugar were calculated from samples harvested in 1993 and applied to prediction for the samples harvested in 1993 and 1994. High-performance liquid chromatography (HPLC) is the method generally used to measure constituent sugars. This method has high accuracy, but requires pretreatment procedures before sample injection onto the HPLC system and has long retention times. HPLC measurement values were used as dependent variables for MLR.
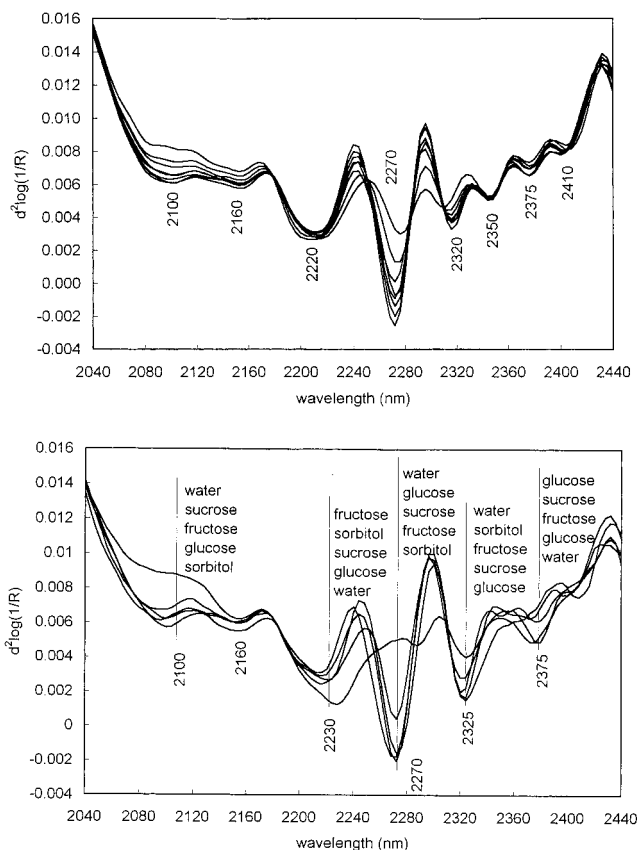
Figure 2. Magnified second-derivative spectra between 2000 and 2400 nm of (a, top) Japanese pear juice in each harvested day and (b, bottom) 10% (w/w) model sugar solutions (i.e., sucrose, glucose, fructose, sorbitol) and water.

Table 2. Average Concentration of Each Constituent Sugar and the Total Sugar Concentration on Each Harvest Day As Measured by HPLC

day[a]	average content (%, w/w)				total (%, w/w)
	sucrose	glucose	fructose	sorbitol	
May 15	0.16	0.26	0.37	2.63	3.42
May 21	0.26	0.89	1.19	4.23	6.58
May 26	0.09	0.99	2.45	5.54	9.07
June 3	0.04	1.20	3.64	4.99	9.87
June 9	0.08	1.22	4.77	4.71	10.79
June 18	0.18	1.11	4.56	3.30	9.16
June 25	0.46	1.32	4.35	2.74	8.87
July 3	2.07	1.57	6.00	2.70	12.35

[a] Harvest date of samples in 1993.

MATERIALS AND METHODS

Samples. A total of 133 fruits of Japanese pears (*Pyrus serotina* var. *culta* 'Kousui') cultivated in 1993 were used for calibration models. About 17 samples were harvested periodically during fruit growth. The harvest days were May 15, 21, and 26, June 3, 9, 18, and 25, and July 3. One day after harvest, the equatorial portion (5-mm thickness) of the flesh was cut off and the juice was squeezed out. Another sample set used for the monitoring test was cultivated in 1994. About 20 samples were harvested periodically. The harvest days were June 4, 11, 19, and 26, and July 4, 12, and 19. All fruits harvested in 1993 and 1994 were selected randomly in a commercial orchard.

Measurement of Near-IR Spectra. The near-IR spectra of juices were measured by diffuse trans-reflectance from 1100 to 2500 nm at 2-nm intervals with a BRAN+LUEBBE Infra-Alyzer 500 equipped with an aluminum cell for the liquid sample. The liquid layer of this cell for data acquisition has a 30-mm diameter and 0.1-mm thickness. The temperature of the juice during measurement was 28 °C. After near-IR

2274 *J. Agric. Food Chem.,* Vol. 44, No. 8, 1996

Tanaka and Kojima

Table 3. Chemical Assignments for Observed Noticeable Absorption Bands

observed[a]	reported[b]	chemical assignment[d]	reported structure
1240−1320[c]			(carbohydrate)
1400−1450	1410	O−H stretching 1st overtone	ROH
	1420	O−H stretching 1st overtone	ArOH
	1440	O−H stretching 1st overtone	sucrose, starch
	1450	O−H stretching 1st overtone	starch, H$_2$O
1490	1480	O−H stretching 1st overtone (intramol. H bond)	glucose
	1490	O−H stretching 1st overtone (intramol. H bond)	cellulose
1530−1630	1528	O−H stretching 1st overtone (intramol. H bond)	starch
	1540	O−H stretching 1st overtone (intramol. H bond)	starch
	1580	O−H stretching 1st overtone (intermol. H bond)	starch, glucose
1684[c]			(carbohydrate)
1724	1725	C−H stretching 1st overtone	CH$_2$
1750[c]			(carbohydrate)
1785	1780	C−H stretching 1st overtone	cellulose
1920−1934	1940	O−H stretching + O−H deformation	H$_2$O
2050−2170	2080	O−H stretching + O−H deformation	sucrose, starch
	2100	2 × O−H deformation + C−O stretching	starch
2250−2284	2252	O−H stretching + O−H deformation	starch
	2276	O−H stretching + C−C stretching	starch
2300−2330	2310	C−H stretching + C−H deformation	CH$_2$
	2323	C−H stretching + C−H deformation	CH$_2$
	2336	C−H stretching + C−H deformation	cellulose
2400−2420[c]			(carbohydrate)
2464	2461	C−H stretching + C−C stretching	starch

[a] Observed absorption band in pear juice. [b] Reported absorption band in the past. [c] Observed in pear juice and model sugar solutions but not reported in the past. [d] These chemical assignments were referred to Osborne and Fearn (1986).
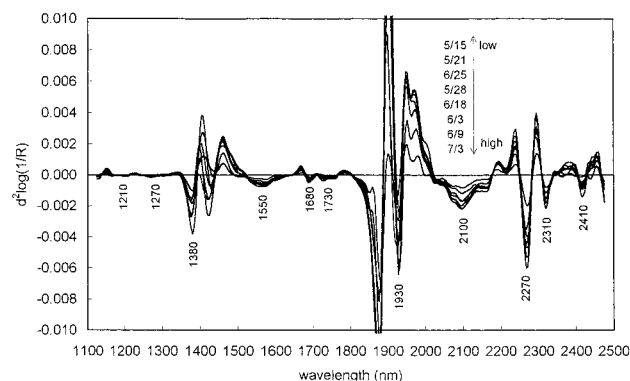

Figure 3. Averaged different spectra of each harvested day subtracted by the spectrum of earliest harvest day (May 15). Indicated wavelength regions have regular order by total sugar concentrations.

Figure 4. Correlation spectrum between total sugar concentration and absorption of each wavelength.

spectra measurement the samples were temporarily stored at −30 °C for chemical analysis (see next section).

Chemical Analysis. Constituent sugar concentrations in Japanese pear juice (namely, sucrose, glucose, fructose, and sorbitol) were measured by HPLC. A 1-mL aliquot of pear juice was diluted six times by adding 5 mL of water, centrifuged at 1000 rpm for 20 min to remove solids, and passed through a 0.45-μm porosity filter. The injected sample volume was 20 μL. The HPLC settings were as follows: column, Shodex SP0810; eluent, water; flow rate, 0.6 mL/min; column temperature, 80 °C; detection, refractive index. To check the reproducibility of the HPLC measurements, each sample was measured twice (Hruschka, 1987). The standard errors of HPLC values (% w/w) of each constituent sugar concentration were as follows: sucrose, 0.004; glucose, 0.012; fructose, 0.035; and sorbitol, 0.039. The values measured by HPLC were of sufficient accuracy for the near-IR analysis.

Multiple Linear Regression (MLR). The IDAS program by BRAN+LUEBBE was used for MLR analysis, which was conducted between the second-derivative spectra and each constituent sugar concentration. The best conditions of the second derivative for each constituent sugar were decided by calculating MLR in every segment and gap. Calibration and prediction sets were prepared for each constituent sugar, and the statistical characteristics of those sets are shown in Table 1. To evaluate fruit maturity, all samples harvested in one day were separated into calibration and prediction sets. The
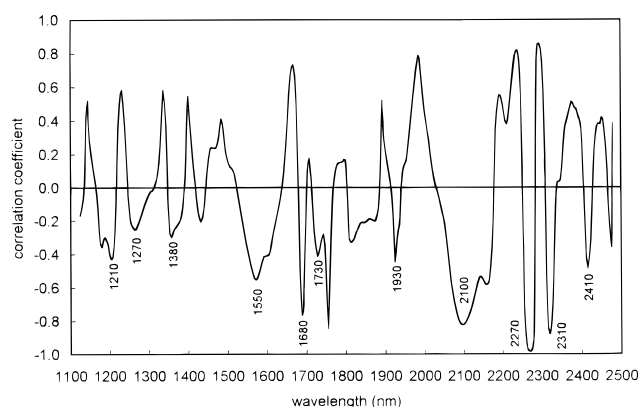
fruits of the Japanese pear in the early growing stage do not have sucrose, which is included in mature fruit; therefore, distribution of sucrose was biased and the mean value is small relative to the range.

Before prediction for the samples harvested in 1994, the calibration equation of each constituent sugar was applied for bias correction. Bias correction means to add or subtract corrected bias values to or from the intercept of each calibration equation calculated by MLR. Twenty randonly selected samples cultivated in 1994 were used for calculating corrected bias values that were averages of differences of measurement values between HPLC and near-IRS.

RESULTS AND DISCUSSION

Near-IR Spectra of Periodically Harvested Japanese Pear Juice. The near-IR original and second-derivative spectra of Japanese pear juice that are the averaged spectra of each harvest day are shown in Figures 1a and 1b, respectively. The pear juice has a high water concentration, so the original spectral shape was similar to a water spectrum. In the original spectra, the clear absorption bands around 1450 and 1940 nm are attributed to water absorption. No other clear absorption band was observed. Vertical differences of absorption among each spectrum were detected

Table 4. Results of MLR for Each Constituent Sugar in Japanese Pear Juice

sugar	wavelength selected							R^a	SEC[b]	SEP[c]	bias
	λ_1	λ_2	λ_3	λ_4	λ_5	λ_6	λ_7				
sucrose	1280	1480	1592	1768	2112	2268[d]	2288	0.97	0.18	0.17	−0.03
glucose	1752	2260[d]	2280					0.90	0.17	0.15	0.00
fructose	1560	1708	2104	2176	2264[d]	2320		0.99	0.24	0.21	0.03
sorbitol	2076	2112	2156	2188	2272[d]			0.98	0.27	0.40	−0.09

[a] R, multiple correlation coefficient. [b] SEC, standard error of calibration. [c] SEP, bias-corrected standard error of prediction. [d] Most significant wavelength in each calibration equation.
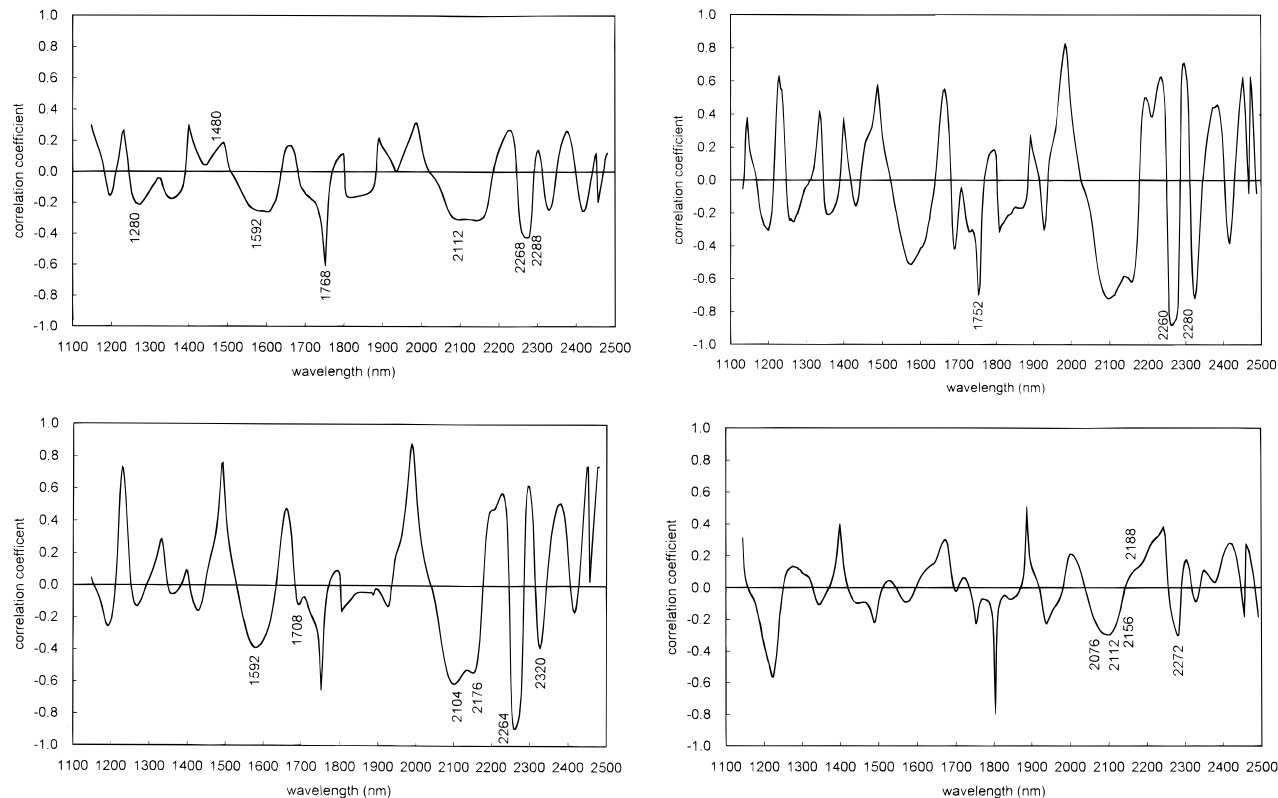

Figure 5. Correlation spectrum between each constituent sugar concentration and absorption of each wavelength; indicated wavelengths were independent variables in each calibration equation: (a, top left) sucrose; (b, top right) glucose; (c, bottom left) fructose; (d, bottom right) sorbitol.

around the water absorption bands. In the second-derivative spectra, some clear absorption bands in addition to water absorption were observed from 1100 to 1300, 1600 to 1800, and 2000 to 2500 nm. The wavelength region from 2000 to 2400 nm had particularly noticeable spectral differences in each spectra. The second-derivative spectra of pear juice and each model sugar solutions magnified from 2000 to 2400 nm are shown in Figures 2a and 2b. These spectra indicate that characteristic absorptions ocurred at the same wavelength regions between pear juice and model sugar solutions. Thus, the slight spectral differences between 2000 to 2400 nm in pear juice spectra depended on sugar absorption.

To identify the spectral vertical difference order in each noticeable wavelength region, averaged constituent sugar concentrations and total sugar concentrations of each harvest day measured by HPLC were assessed (Table 2). The ascending order of total sugar concentration among each harvested day was 5/15, 5/21, 6/25, 5/28, 6/18, 6/3, 6/9, and 7/3, and the spectral vertical order of absorption bands at 2100, 2270, and 2310 nm depended on this order. These results indicate that changes in sugar concentration with growth of the fruit of Japanese pear strongly influence near-IR spectra from 2000 to 2400 nm.

To clarify the absorption difference between harvest days, the second-derivative difference spectra based on the earliest harvest spectrum (5/15) were examined (Figure 3). These spectra show some clear differences in the wavelength regions around 1210, 1270, 1550, 1680, 1730, 1930, 2100, 2270, 2310, and 2410 nm. As shown in Table 3, the reported chemical assignment around these absorption bands (Osborne and Fearn, 1986) and the noticeable wavelength regions were consisted with the absorption bands of C−H, O−H, C−O, and C−C. The correlation spectrum between total sugar concentration and each absorbance from 1100 to 2500 nm is shown in Figure 4. In the second-derivative spectrum, peak direction was upside down compared with the raw spectra; hence, the wavelength region highly related to total sugar concentrations has negative values. The most significant wavelength with regard to the combined absorption band of an O−H stretching and C−C stretching mode (Osborne and Fearn, 1986) and whose correlation coefficient was −0.98 was 2270 nm. Other wavelength regions with regular vertical order to the total sugar concentration also had high correlations.

Results of MLR. The best conditions of the second derivative for each constituent sugar [segments (nm)/gaps (nm)] were 36/12 for sucrose, 32/16 for glucose, 24/

2276 *J. Agric. Food Chem.,* Vol. 44, No. 8, 1996
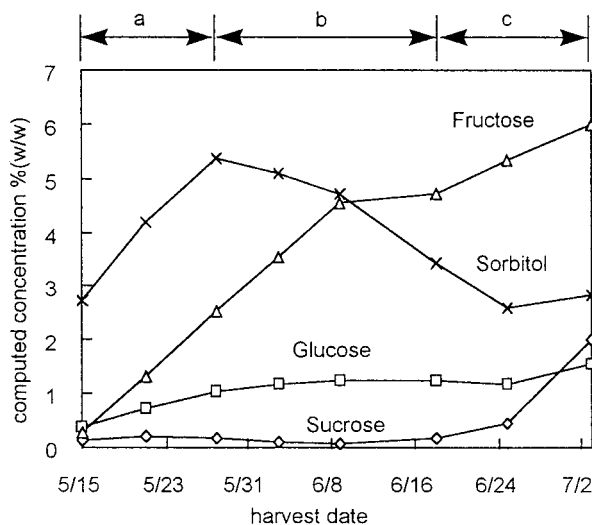
Tanaka and Kojima

Figure 6. Change of each constituent sugar concentration measured by near-IRS for Japanese pear cultivated in 1993: (a) cell division stage; (b) thickening growth stage; (c) ripening growth stage.

24 for fructose, and 32/12 for sorbitol. The results of MLR, shown in Table 4, indicate that constituent sugar concentrations could be measured by regression equations with three to seven wavelengths. The optimal number of wavelengths as independent variables in the calibration equation was given by the bias-corrected standard error of prediction (SEP) before overfitting. The correlation coefficients (R) of sucrose, glucose, fructose, and sorbitol were 0.97, 0.90, 0.99, and 0.98, respectively with SEP values of 0.17, 0.15, 0.21, and 0.40, respectively. The most significant wavelengths in each calibration equation determined by the t test were 2268 nm for sucrose, 2260 nm for glucose, 2264 nm for fructose, 2272 nm for sorbitol. These wavelengths are the same absorption bands that had the highest correlation coefficient with total sugar concentration. The t test values were calculated as the ratio of the regression coefficient to the standard deviation of the coefficient and they indicate the degree of contribution of each wavelength to the calibration calculation (BRAN+LUEBBE, 1989).

The correlation spectra between each constituent sugar concentration and each absorbance from 1100 to 2500 nm are shown in Figure 5 (a−d). The independent variables of each calibration equations are indicated. Every correlation spectra has some broad peaks that had a high correlation coefficient with each constituent sugar concentration. The correlation spectra shape of sucrose, glucose, and fructose but not sorbitol, are similar to each other. Every important wavelength of each calibration equation existed in broad peaks.

Evaluation of the Ripeness of the Fruit. Results of prediction with each sampling day in 1993 are shown in Figure 6. Young fruits harvested on 5/14 took 30 days after pollinating to reach a diameter of ~30 mm. In this stage, sorbitol concentration was the highest and sucrose concentration was the lowest. Sorbitol concentration increased until 5/28 and reached >6% (w/w). After 5/28, sorbitol concentration started decreasing, indicating that the fruit growth stage changed from the cell-division stage to the thickening-growth stage (Endo, 1973; Yamaki et al., 1979a). After 6/18, sucrose concentration increased gradually, indicating that the ripening growth stage was beginning. Commercial harvest for ripe fruits started around 7/8, which was

Table 5. Statistic Values of 20 Samples Cultivated in 1994 for Bias Correction[a]

sugar	range	mean	SD[b]	bias	SEP[c]
sucrose	0.08−1.02	0.25	0.22	3.11	0.29
glucose	0.74−1.03	0.85	0.07	0.46	0.17
fructose	2.13−3.67	2.89	0.34	−0.44	0.49
sorbitol	1.81−3.08	2.45	0.35	−0.19	0.33

[a] Unit used, % (w/w). [b] SD, standard deviation. [c] SEP, Bias-corrected standard error of prediction.
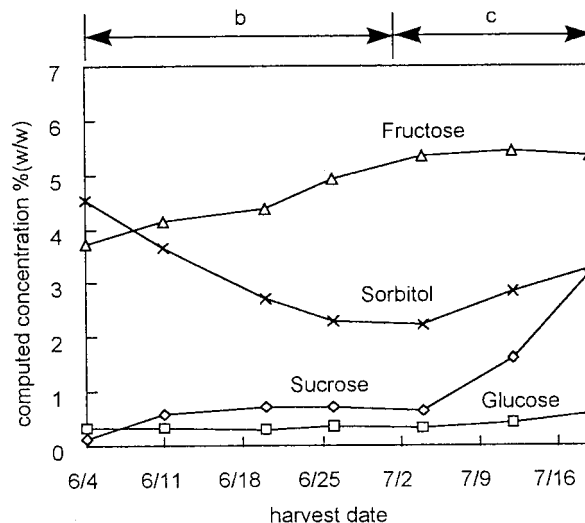

Figure 7. Change of each constituent sugar concentration measured by near-IRS for Japanese pear cultivated in 1994.

~3 months after pollination. In the ripening stage, fruit size was constant and the diameter was ~100 mm. In this stage, fructose attained the highest concentration (>5% w/w), followed by sorbitol at 2.5% (w/w), and sucrose and glucose at 1% (w/w).

The statistical characteristics of 20 samples for bias correction in 1994 are shown in Table 5. The SEP and bias value for the 1994 samples were calculated between HPLC and near-IRS, and their range of SD were less than those of the calibration sets of 1993. The SEP of each constituent sugar was bigger in 1994 than in 1993, but each calibration equation had sufficient accuracy. The ratios between the SD of the calibration set in 1993 and the SEP of 20 samples were 2.52 for sucrose, 2.24 for glucose, 3.71 for fructose, and 3.79 for sorbitol.

The results of prediction in 1994 determined by bias-corrected calibration equations, are shown in Figure 7. In the first sampling day (6/4), sorbitol concentration was the highest and started decreasing. Thus, the phase after 6/4 was the fruit-thickening growth stage. After 7/3, sucrose concentration increased gradually, and the thickening-growth stage extended to 7/3, when the ripening stage began. Commercial harvest for ripe fruits started around 7/10, at which time the fruit diameters were ~85 mm. In this stage, the sugar with the highest concentration was fructose at <5% (w/w), the second highest was sorbitol at 3% (w/w) and next highest were sucrose at 1.5% (w/w) and glucose at 0.3% (w/w). During July and August, 1994, the very hot temperatures and less than optimal rainfall caused serious water deficiency. The reason the fruit diameters in 1994 were smaller than in 1993 was that the water supply for fruits was not enough during the fruit-thickening growth stage.

The patterns of changes for each constituent sugar concentration determined by near-IRS in 1993 and 1994 were consistent with those reported by Yamaki et al.

Near-IR Monitoring of Pear Growth

J. Agric. Food Chem., Vol. 44, No. 8, 1996 **2277**

(1979a). Therefore, near-IRS can be used for evaluating the growth phase of Japanese pear juice effectively and would even be more accurate estimation method when combined with other harvest indices, such as fruit appearance. For practical use in the orchard with intact fruits, near-IRS needs more investigation.

ACKNOWLEDGMENT

LITERATURE CITED

Bellon, V.; Boisde, G. Remote near infrared spectrometry in the food industry with use of silica and fluoride glasses fibers. *Proceedings of SPIE 89*; Los Angeles, CA, 1989.

Biale, J. B. Growth, maturation and senescence in fruits. *Science* **1964**, *146*, 880−888.

Burg, S. P.; Burg, E. A. Role of Ethylene in fruit ripening. *Plant Physiol.* **1962**, *37* (*1*), 179−189.

BRAN+LUEBBE. Section 3. Operation Manual; BRAN+ LUEBBE, 1989.

Dull, G. G.; Birth, G. S.; Smittle, D. A.; Leffler, R. G. Near infrared analysis of soluble solids in intact cantaloupe. *J. Food Sci.* **1989**, *54* (*2*), 393−395.

Endo, M. Studies on the daily change in fruit size of the Japanese pear. *J. Jpn. Soc. Hortic. Sci.* **1973**, *42* (*2*), 91−103.

Giangiacomo, R.; Dull, G. G. Near infrared spectrophotometric determination of individual sugars in aqueous mixtures. *J. Food Sci.* **1986**, *51* (*3*), 679−683.

Hruschka, W. R. *Data Analysis: Wavelength Selection Methods. Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P.; Norris, K., Eds.; AACC: St. Paul, Mn, 1987.

Kawano, S.; Takehara, K.; Sato, T.; Iwamoto, M. Determination of sucrose content in sugarcane juice by near-IR spectroscopy. *The Proceedings of the Third International Conference on Near Infrared Spectroscopy*; Agricultural Research Center Publishing: Gembloux, Belgium, 1991.

Kawano, S.; Fujiwara, T.; Iwamoto, M. Nondestructive determination of sugar content in satsuma mandarin using near infrared (near-IR) transmittance. *J. Jpn. Soc. Hortic. Sci.* **1992a**, *62* (*2*), 465−470.

Kawano, S.; Watanabe, H.; Iwamoto, M. Determination of sugar content in intact peaches by near infrared spectroscopy with fiber optics in interactance mode. *J. Jpn. Soc. Hortic. Sci.* **1992b**, *61* (*2*), 445−451.

Lanza, E.; Li, B. W. Application for near infrared spectroscopy for predicting the sugar contents of fruit juices. *J. Food Sci.* **1984**, *49*, 995−998.

Murray, I.; Williams, P. C. *Chemical Principles of Near-Infrared Technology. Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P.; Norris, K., Eds.; AACC: St. Paul, MN, 1987.

Osborne, B. G.; Fearn, T. *Theory of Near Infrared Spectrophotometry. Near Infrared Spectroscoy in Food Analysis*; Longman Science & Technical: New York, 1986.

Williams, P. C.; Norris, K. *Near-Infrared Technology in the Agricultural and Food Industries*; AACC: St. Paul, MN, 1987.

Yamaki, S.; Kakiuchi, N. Changes in hemicellulose-degrading enzymes during development and ripening of Japanese pear fruit. *Plant Cell Physiol.* **1979**, *20* (*2*), 301−309.

Yamaki, S.; Kajiura, I.; Kakiuchi, N. Changes in sugars their related enzymes during development and ripening of Japanese pear fruit. *Bull. Fruit Tree Res. Stn.* **1979a**, *A6*, 15−26.

Yamaki, S.; Machida, Y.; Kakiuchi, N. Changes in cell wall polysaccharides and monosaccharides during development and ripening of Japanese pear fruit. *Plant Cell Physiol.* **1979b**, *20* (*2*), 311−321.