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REVIEW
New Ways to Evaluate the Quality of Vegetables Using Instruments

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Abstract

This paper reviews new methods of evaluating the quality of vegetables, which included physical methods, ion-selective electrode (ISE), liquid chromatography (LC) (high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC)), gas chromatography (GC), visible (Vis) spectroscopy, near-infrared (NIR) spectroscopy and VIS-NIR spectroscopy. Moreover, penetrability, juiciness, nitrate ions, lycopene, glucosinolate (GSL), isothiocyanate (ITC) determination, rapid total dietary fiber (TDF) determination and non-destructive determination were also described.

Discipline: Postharvest technology
Additional key words: browning, determination, intact, internal disorder, soluble solids

Introduction

Vegetable quality has been evaluated by physical and chemical properties, which are mainly measured using instruments with objectivity and many such instruments have been applied to evaluate vegetable quality. Developing an exact and proper destructive method of evaluating quality involves a low-cost or non-destructive method for end users.

Methods of measuring the quality of agricultural products have evolved from macro- to micro scale, or from destructive- to non-destructive methods. For example, liquid chromatography (LC) has evolved into high-performance liquid chromatography (HPLC) and subsequently ultra-performance liquid chromatography (UPLC). UPLC can determine compound quality with less liquid sample and shorter times. The method of measuring absorbance or firmness has evolved from destructive- to a non-destructive method for practical use.

The instruments have also evolved to become more compact. For example, an ion-selective electrode (ISE) or near-infrared (NIR) spectrophotometer has evolved from bench to portable size with a lower price for practical use.

Under the above circumstances, individual cases involving the measurement of vegetable quality have been tackled as mentioned below, with new methods shown in Table 1. The instruments were categorized in terms of physical methods, ISE, LC (HPLC, UPLC), gas chromatography (GC), Visible (VIS) spectroscopy, NIR spectroscopy and VIS-NIR spectroscopy, whereupon penetrability, juiciness, nitrate ions, lycopene, glucosinolate (GSL), isothiocyanate (ITC) determination, rapid total dietary fiber (TDF) determination and non-destructive determination were also described.

Physical methods

Minimally-processed fresh or fresh-cut vegetable production is important, in developed as well as advanced countries, and raw cabbage and its cut products are often consumed. Sweetness, strength (penetrability), and juiciness determine cabbage quality and sweetness can be evaluated by measuring sugar content. However, penetrability had been measured for a specific or individual leaf and not for a whole cabbage simultaneously. No juiciness measurements using an instrument had been reported.

The penetrability of a half-cut cabbage was measured using an instrument (Autograph, AGS-100B, Shimadzu, Japan) equipped with a 150 mm-long needle, whereupon a cabbage cut vertically in half was examined. The juiciness was then measured using the same instrument equipped with a metal disk (57 mm in diameter) and the cut cabbage was pressed with the disk at constant pressure. Both soft,

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Table 1. Categorized new methods of evaluating vegetable quality

Physical method	Penetrability of a half cut-cabbage 7).
	Juiciness measurement of cabbage with constant pressure using an instrument 7).
	Non-destructive method of determining harvest time of melons on tree 17).
Ion-selective electrode (ISE)	Determining nitrate ions in vegetable juice without dilution 6,8).
	(Reference values determined using ion chromatography (IC) 25).)
Liquid chromatography (LC)	Determination of nitrate ions without overlapping nitrate and citrate ions using IC 25).
	Direct analysis of 4-methylthio-3-butenyl glucosinolate (MTB-GSL) in the Japanese radish using high-performance liquid chromatography (HPLC) 10).
	Determination of lycopene in tomatoes using HPLC 11) or ultra-performance iquid chromatography (UPLC).
Gas chromatography (GC)	Rapid determination of 4-methylthio-3-butenyl isothiocyanate in Japanese radishes and L-sulforaphane in broccoli sprouts using diethyl ether as a direct extraction solvent13).
Optical methods	
(1) Visible (VIS) spectroscopy	Rapid determination of lycopene in tomatoes with diethyl ether/methanol (7:3,v/v) 11).
(2) Near-infrared (NIR) spectroscopy	Rapid determination of total dietary fiber (TDF) in dehydrated vegetables 19).
	Non-destructive determination of soluble solids in melons5,18)*, tomatoes* strawberries3)* or compact watermelons 20)*.
	Non-destructive water-soaked symptoms or browning detection in melons 23)*.
	Non-destructive inner scale disorder(Shingusare in Japanese) detection in onions(*In press*).
(3)VIS-NIR spectroscopy *	Non-destructive determination of nitrate ions in Japanese radishes 22), leaf stalk of Qing gin cai (*Brassica chinensis*) 14) or stem end of lettuces.
	Non-destructive determination of lycopene in tomatoes 15).
	Non-destructive outer scale disorder (Hadagusare in Japanese) detection in onions 9).

* Application of non-contact spectral measurement of interactance mode that can improve the accuracy of non-destructive determination.

juicy and hard and less juicy cabbages were examined, and the instrument confirmed that the former were softer and juicier than the latter. In addition, large-cut cabbages were less juicy than small-cut varieties. When penetrability was measured, the kgf value of the vertical axis increased and the cabbage midrib with hard characteristics could be identified on the time-kgf chart when penetrability was measured, meaning the instrument could be used to measure penetrability and juiciness[7].

The harvest time of a melon is usually decided by days after crossing, but such decisions are not easy when the cultivation conditions vary. Conversely, the firmness of a harvested netted melon cultivar can be measured non-destructively, whereby the transmission velocity (m/s) of sound was measured using a non-destructive firmness tester. The softer the fruit, the slower the transmission velocity[29].

When the growing fruit was mature on the tree and particularly when it was sunny, the transmission velocity was slower. When the transmission velocity was twice measured below 80 m/s, the sucrose content amounted to more than 8.5%, indicating maximum content. Conversely, the total content of glucose and fructose was virtually constant. Consequently, it was concluded that the transmission velocity could be used to determine the harvest time of melon fruit on tree non-destructively[17].

Ion-selective electrode (ISE)

The nitrate-ion content of vegetables is related to food safety and several studies have addressed a potential link between nitrate-ion exposure and childhood type 1 insulin-dependent diabetes mellitus. The nitrate ions people consume are 50-90% derived from vegetables and the EU has a guideline for nitrate ions in vegetables and there are numerous methods by which nitrate ions can be determined. Simple and low-cost methods using reflectance mode (RQflex, Merck, Germany) or ISE can be performed in the field, while ion chromatography (IC) or capillary electrophoresis (CE) are possible in a laboratory. However, the nitrate-ion concentrations determined by each of the 4 methods may differ. Conversely, because only the ISE can measure undiluted vegetable juice of all 4 methods, there is no error of dilution. In addition, applicability to vegetable samples is inadequate due to the many vegetables while nowadays, reference values can be determined using not a colorimetric but a reliable IC method.

First, the determined nitrate-ion concentration was compared using ISE, RQflex, CE and IC. When the dilution rate of the spinach and Japanese radish juice was fixed 100-fold, the concentration was next order, ISE ≧ RQflex > CE =IC. The ISE- and RQflex-determined values of 100-fold dilution exceeded those of 50-fold dilution, respectively, which mean that the nitrate-ion concentration determined using ISE or RQflex can be changed by diluting. In addition, an old ISE could easily be measured as higher than a new ISE within the high concentration range and the response of the old ISE was slow[21], which means the old ISE is likely to have higher values. However, as mentioned above, only ISE of the 4 methods can measure the juice of vegetables without dilution, so ISE was continuously used for experiments without dilution of vegetable juice.

A portable ISE developed by Horiba, Ltd., Japan was evaluated against an aqueous solution of potassium nitrate and vegetable juice. The ISE exhibited a linear dynamic range from 20 to 6000 ppm nitrate ions in standard aqueous solutions of potassium nitrate, while for nitrate-ion spiked samples of raw vegetable juice, the recovery percentage consistently approached 100%. Furthermore, the results determined by ISE without dilution were essentially identical to those determined by IC mentioned below, exhibiting a correlation coefficient of R =0.997 (n =7). The use of an ionic strength adjustor was deemed unnecessary in these experiments, which facilitates implementation in practical applications[8]. The instrument is currently commercially available as a compact nitrate ions meter, B-343, Horiba Ltd., Japan. Nitrate - ISE needs to shorten measuring times and improve selectivity for nitrate ions in some vegetables[6].

Liquid chromatography (LC)

1. Ion chromatography (IC)

Nitrate-ion concentrations in leafy *Cruciferae* and spinach are high, while spinach also contains a relatively high level of oxalate, the uptake of which may cause urinary stones. Metabolism of nitrate, oxalate and ascorbic acid is inter-related, as are cations and other anions. This means it is important to quantify anions, including the other major organic acids (malate and citrate) in spinach. IC, for which a low-conductivity eluent is used to detect electrical conductivity is intended for the simultaneous determination of organic and inorganic anions in vegetable samples, while spinach harvested in winter sometimes contains high levels of organic acids, though the electrical conductivity detector is not sensitive to organic acids. Accordingly, nitrate-ion determination might be affected by nitrate and organic ions overlapping.

Only the IC-determined nitrate-ion concentration of the 4 methods mentioned in ISE above was unaffected by dilution of vegetable juice. However, the conditions of IC[31] could be applied when few citrate ions were detected because the peak of the nitrate ions overlapped that of the citrate ions. When the pH of the eluent changed from 4.0 to 4.3, citrate and nitrate ions were separated without interference[25]. This method could be useful and practical for vegetables[6].

2. 4-Methylthio-3-butenyl glucosinolate (MTB-GSL)

When raw crucifers are grated, chewed, or chopped, glucosinolates (GLSs) come into contact with endogenous myrosinase (thioglucoside glucohydrolase EC 3.2.3.1), and breakdown products, including isothiocyanates (ITCs), nitriles, and thiocyanates, are released. Many methods of analyzing GSLs have been developed. Desulfo-GSLs are analyzed using HPLC, trimethylsilyl derivatives using GC, and aglycon using GC or spectrophotometry. Accordingly, the methods need chemical modification by enzymatic treatment and/or silylation and are time-consuming. Now however, a simple and direct method for analyzing GSLs content is available, which utilizes paired-ion chromatography using tetraalkylammonium ions as counter ions. Moreover, replacing the counter tetradecyl ammonium (TDA) ions with ammonium ions permits direct bioassay, mass spectrometry and ^{1}H-nuclear magnetic resonance spectroscopy. However, no direct analytical method for 4-methylthio-3-butenyl GSL (MTB-GSL) in the Japanese radish, which is one of the main vegetables in Japan, has yet been reported.

To isolate and measure intact MTB-GSL in Japanese radish, we applied a paired-ion chromatography method using TDA ions as counter ions. MTB-GSL was isolated as an ammonium salt from the root of a Japanese radish and identified based on myrosinase treatment, aglycon detection, negative fast atom bombardment-mass spectra, and a UV spectrum. When the prepared standard solution was added to Japanese radish extracts, recovery was almost 100%[10].

3. Lycopene

Trans-lycopene (lycopene), the major carotenoid found in pink or red tomatoes, imparts a characteristic red color. Lycopene is also an indicator of maturity, which is critical for palatability and also attracts our attention as a functional compound. Some extraction solvents were used to determine lycopene in tomatoes. However, we were unsure which solvent was best to extract lycopene in tomatoes because their extraction efficiencies had not been compared. On the other hand, lycopene is unstable in an HPLC system, meaning proper lycopene determination using HPLC might be described with the selected solvent. Moreover, a new rapid determination method for lycopene might be also developed with the selected extraction solvent.

The best extraction efficiency of lycopene in tomatoes was achieved by employing diethyl ether/methanol (7:3, v/v) using HPLC. On the other hand, when a standard lycopene solution was injected into the HPLC, the rate of the peak area varied among C18 columns. For the low rate of a lycopene peak, part of the lycopene appeared decomposed and the determined lycopene value was higher; hence the use of a C18 column with a high rate of lycopene peak is recommended.

A rapid determination method using a VIS spectrophotometer was developed using the best extraction solvent at 505 nm and an absorption coefficient of 3150$\%^{-1}$ cm^{-1}. The correlation coefficient (R) between the values determined using the HPLC versus the rapid determination method was 0.987 (n =32)[11].

When ammonium acetate was added to an eluent of HPLC or UPLC (Waters corporation, USA), the peak area of not only the lycopene but also other carotenoids increased (Tables 2, 3), while R between the values determined using HPLC versus the rapid determination method was 0.999 (n=9), reflecting the perfect recovery of lycopene without bias and slope. R between the values determined using UPLC versus the rapid determination method was also 0.997 (n=9) (Fig. 1). However, the UPLC results showed only high recovery of lycopene, so the lycopene determination in tomatoes using UPLC, which would be expected as a rapid and accurate method, was particularly lacking in stability.

Table 2. Influence of ammonium acetate concentration in eluent of UPLC* on the peak area or peak area rate (%) of lycopene

Conc.(w/v%)	Peak area	Rate of peak area (%)
0.00	373599 ± 81780	57.9 ± 10.2
0.01	731204 ± 92730	70.9 ± 3.7
0.10	1213022 ± 33400	96.9 ± 0.5
0.20	1170229 ± 283173	97.0 ± 0.3

*The UPLC analysis conditions were as follows.
Eluent: methanol 100% base, 0.21 mL/min; Column: Acquity UPLC™ BEH Shield RP18, particle size 1.7 μm, i.d. 2.1 mm, length 150 mm (Waters corporation, USA), 25°C; Detection: 450 nm.
3 μL of lycopene dissolved in acetone/hexane (4:6, v/v) was injected three times (n=3), respectively.

Table 3. Influence of ammonium acetate (AA) concentration (0, 0.1%(w/v)) in eluent of UPLC[1)] on the peak area of carotenoids

Carotenoid[2)]	Peak area (AA 0%)	Peak area (AA 0.1%)
Violaxanthin	83894 ± 2143	92564 ± 6012
Lutein	33024 ± 1358	110570 ± 5333
Zeaxanthin	21205 ± 2033	116889 ± 1839
α-carotene	45510 ± 3301	154544 ± 8202
β-carotene	20770 ± 3161	156936 ± 12646

[1)] The UPLC analysis conditions were as follows.
Eluent: methanol 100% base, 0.21 mL/min; Column: Acquity UPLC™ BEH Shield RP18, particle size 1.7 μm, i.d. 2.1 mm, length 150 mm (Waters corporation, USA), 25°C; Detection: 450 nm.

[2)] 3 μL of reagents (butch No.viol-127, lut-119, zea-130, alfa-120 and beta-118 of DHI Laboratory products (Denmark)) was injected three times (n=3), respectively.

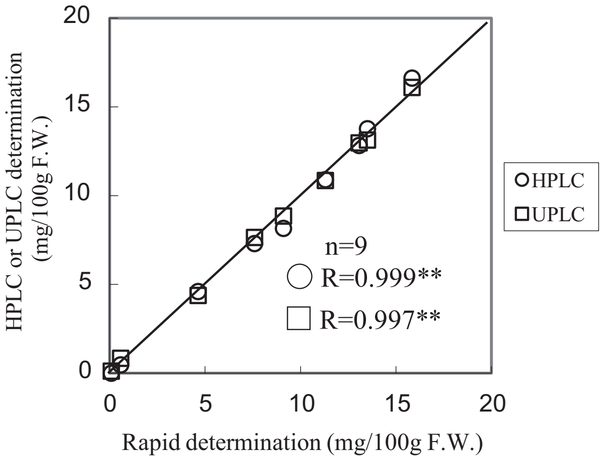


Fig. 1. Plots of lycopene values in tomatoes

The HPLC analysis conditions were as follows. Eluent: ammonium acetate 0.01% (w/v) in methanol 100%, 1.0 mL/min; Column: Mightysil RP-18 GP 150-4.6 (Kanto Kagaku, Japan), 25°C; Detection: 450nm.

The UPLC analysis conditions were as follows. Eluent: ammonium acetate 0.1% (w/v) in methanol 100%, 0.21 mL/min; Column: Acquity UPLC™ BEH Shield RP18 2.1×150 mm (Waters corporation, USA), 25°C; Detection: 450 nm.

As mentioned above, LC methods sometimes involve the low recovery of carotenoids. Accordingly, a means of rapid determination using VIS spectroscopy was used when the non-destructive method was developed, as mentioned below.

LC methods including HPLC and UPLC may be considered exact and proper, but their suitability should still be confirmed.

Gas chromatography (GC)

The anticarcinogenic property of crucifers had been elucidated and isothiocyanates (ITCs) are responsible for protection against carcinogens. 4-Methylsulfinylbutyl ITC (L-sulforaphane) in broccoli sprouts is famous for strongly inducing phase-2 detoxication enzymes, while 4-Methylthio-3-butenyl ITC (MTB-ITC) in Japanese radish sprouts called kaiware-daikon in Japan is also anticarcinogenic. However, the determination methods were time-consuming and few preharvest effects on ITCs in sprouts were reported. Accordingly, the naturally occurring preharvest effects on both ITCs content were researched with a simple and rapid extraction method.

The extraction method uses diethyl ether as a direct-extraction solvent with vegetable juice[4,13]. In broccoli and Japanese radish sprouts, varieties and harvest time affected both L-sulforaphane and MTB-ITC content, respectively. The earlier the harvest, the greater the content of both ITCs.

A greening period had no effect on L-sulforaphane content and also appeared to have no effect on MTB-ITC content. GC and GC-mass spectrometry analysis of broccoli sprouts extract revealed 4-methylthiobutyl ITC and 4-methylthiobutyl nitrile as the main components that resembled the structure of L-sulforaphane. L-sulforaphane content was lower than MTB-ITC content though exogenous myrosinase treatment after the microwave treatment of broccoli sprouts increased L-sulforaphane content about twentyfold. As mentioned above, the low content (μM / 100mL of sprout juice=μmole / 100mL of sprout juice) of L-sulforaphane in broccoli sprouts should be considered[13].

NIR spectroscopy

The potential of NIR spectroscopy for non-destructive quality evaluation in horticultural products was reported, with early applications mainly for soluble solids (Brix), used to indicate sweetness in harvested fruit. However, the potential of NIR spectroscopy for non-destructive evaluation of Brix in strawberries and watermelons, and internal disorder in vegetables had not been reported. Determining Brix in intact watermelons using NIR spectroscopy might be difficult due to the thick and hard rind. Conversely, an improved method of the accuracy of non-destructively determined Brix values might also emerge.

To enable vegetable quality to be determined rapidly or non-destructively, absorption spectra of vegetable samples were used as independent variables of multiple linear regression (MLR) analysis and a simple calibration for each determination was applied because of the difficulty in interpreting NIR models when dealing with models involving many wavelengths[26].

1. Dietary fiber content of dehydrated vegetables

Total dietary fiber (TDF) also attracts our attention as a functional compound, but processes used to determine TDF are very time-consuming. An NIR reflectance spectrophotometric method might enable TDF in dehydrated vegetables to be rapidly assayed.

Dehydrated vegetables were milled, and TDF was measured by the AOAC (985.29) enzymatic-gravimetric method, with values ranging from 8.41 to 37.19% of the dry weight. The milled vegetables were scanned from 1100 to 2498 nm using an NIRSystems 6500 spectrophotometer (Foss, Italy). The spectra (log1/R) were converted to the second derivative spectra (d^2 log 1/R), whereupon the selected wavelengths in the calibration equation were d^2 log 1/R at 2244, 2214, 2392 and 1172nm and the multiple correlation coefficient (MR) of the calibration sample set (n=66) was 0.94. A validation sample set (n=22) was effectively predicted. However, other samples (n=27) generated bigger differences between the value of AOAC-determined TDF and that of NIR-calculated TDF; namely those with viscosity, high TDF content, brown dehydrated vegetables, making it impossible to observe peak tops in the vicinity of 2248nm, highly NIR-calculated TDF values (exceeding 35% or so) or grown under dark conditions. As mentioned above, if the applied samples are distinguished, NIR spectroscopy can be used to rapidly estimate the TDF content of dehydrated vegetables[19].

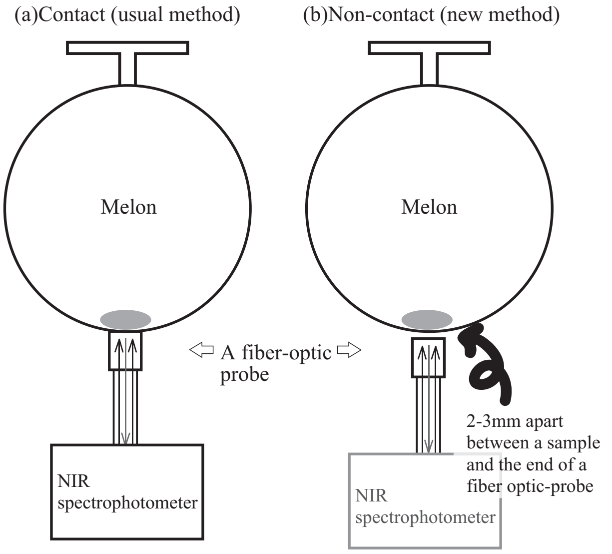


Fig. 2. 'Contact mode' and 'Non-contact mode' in interactance modes
A black arrow means an illuminated light to a sample (melon).
A grey arrow means light collected by a spectrophotometer.

2. Soluble solids (Brix%) in intact vegetables

(1) Melons

To measure the NIR absorption spectrum (log1/R), each melon was manually placed 2-3 mm away from the end of a fiber optic probe of instruments ('Non-contact interactance mode' of spectral measurement) so that the blossom end of melon was centered (Fig. 2 (b)). Though the equator of the melon has many measuring points, the blossom end has only one. Moreover, the flesh under the blossom end is thinner and riper than the other part of the melon. The spectra were converted to the second derivative spectra (D^2 log 1/R).

Using an NIRSystems 6500 spectrophotometer (FOSS, Italy), the 'Non-contact interactance mode' could determine Brix more accurately compared to the usual 'Contact interactance mode' (Fig. 2)[18]. D^2 log 1/R at 828, 856, 876 and 902 nm were selected as independent MLR calibration variables for 'Non-contact interactance mode', while other researchers selected in the vicinity of 880 and 905 nm for non-destructive Brix determination in melons, onions, peaches and nectarins. D^2 log 1/R at 832 and 856 converged at zero values. For practical use, a low-cost, high-performance and portable instrument (K-BA100, Kubota Corporation, Japan, Figs. 2(b); 3(b)) was developed[27]. The calibration selected similar independent variables available for growing[16], harvested, netted, non-netted and different flesh-colored melons[5].

(2) Strawberries

The optical absorption spectrum of each strawberry was measured using an NIRSystems 6500 spectrophotometer equipped with a fiber optic probe ('Contact interactance mode') (Figs. 2 (a); 3(a)). D^2 log 1/R in the vicinity of 907 and 882 nm were selected as independent MLR calibration variables for Brix. The main constituents of Brix were sucrose, glucose and fructose, the absorption of which was in the vicinity of 908nm[3].

Non-destructive determination of Brix in strawberries was performed using both bench-type and portable instruments (NIRSystems 6500, FOSS, Italy and K-BA100, Kubota, Japan), the requirements for which were discussed with a fiber optic probe in interactance mode, though the diameters of the 2 probes differed (Fig. 3). The portable instrument appeared more useful than the bench-type instrument because the former could determine Brix more accurately. However, the portable instrument must be improved to measure the spectra of very small strawberries properly and the bench-type instrument also, to estimate Brix more accurately[24].

For practical use, a method of calibrating Brix in whole strawberries was developed using the portable instrument (K-BA100, Kubota Corporation, Japan[27] (Figs. 2(b); 3(b))). D^2 log 1/R at 832, 884 and 902 nm were selected as independent MLR calibration variables (Fig. 4). The calibration can be written as follows:

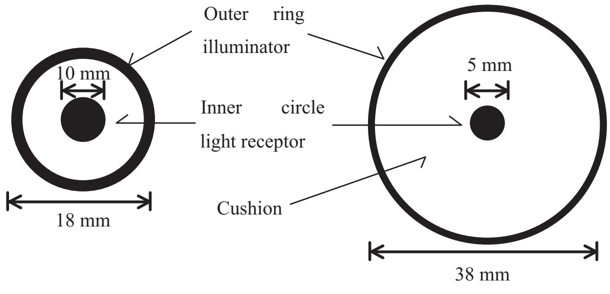
$$\text{Brix in an intact strawberry} = 8.53 + 44300 \times D^2 \log 1/R_{832} + 219000 \times D^2 \log 1/R_{884} - 70600 \times D^2 \log 1/R_{902}$$

(3) Watermelons

The absorption spectrum of each compact watermelon was measured using a K-BA100 spectrophotometer (Kubota Corporation, Japan) equipped with a fiber optic probe ('Non-contact interactance mode' (Figs. 2(b); 3(b))). Key wavelengths, 902 and 872 nm for non-destructive Brix determination were selected as independent MLR calibration variables. For 23 compact watermelons of the calibration sample set, MR was 0.87. In addition, d^2 log 1/R at 902 nm declined with increasing fruit thickness and it was concluded that Brix in compact watermelons could be determined non-destructively using NIR spectroscopy[20]. It is also available for practical use.

(4) Tomatoes

The absorption spectrum of each tomato was measured using a K-BA100R spectrophotometer (Kubota Corporation, Japan) equipped with a fiber optic probe ('Non-contact interactance mode' (Figs. 2(b); 3(b))).



(a) NIRSystems 6500 (b) K-BA100 or 100R

Fig. 3. The end of two fiber optic probes (sample stages) of NIR instruments

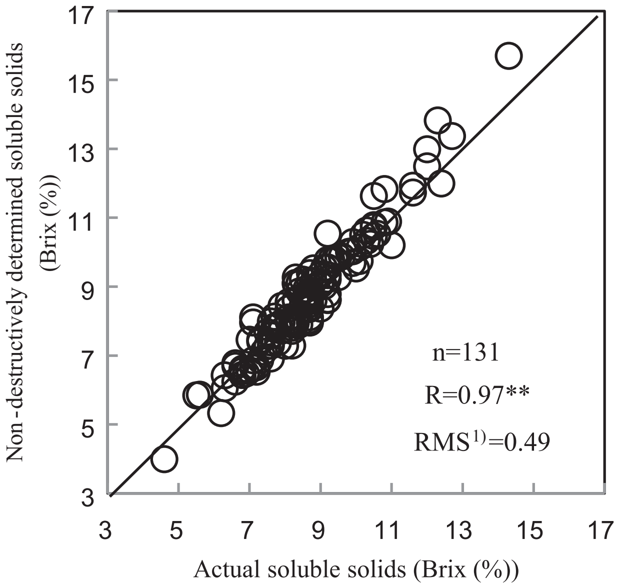


Fig. 4. Plots of actual and non-destructively determined soluble solids (Brix (%)) in whole strawberries

The average of two non-destructively determined soluble solids values was plotted per strawberry.

1) RMS(Root Mean Square)= $\sqrt{\dfrac{\Sigma\,(X-Y)^2}{n}}$

n=samples, X=actual Brix (%),
Y=non-destructively determined Brix (%)

For practical use, Brix was calibrated in whole tomatoes using the instrument. D^2 log 1/R at 856, 876 and 902 nm were selected as independent MLR calibration variables (Fig. 5). The calibration can be written as follows:

$$\text{Brix in an intact tomato} = 6.77 - 49400 \times D^2 \log 1/R_{856} + 315000 \times D^2 \log 1/R_{876} - 125000 \times D^2 \log 1/R_{902}$$

Certain optical measurement modes of the absorption spectrum are available using a spectrophotometer, while transmission and interactance modes are often used to determine Brix non-destructively in fruit. Which mode is more accurate for non-destructively determined Brix values using NIR spectroscopy? Many people tend to say the transmission mode.

The accuracy of the 'Non-contact interactance mode' was compared with that of the transmission mode using NIR spectroscopy and the transmission spectrum of each tomato was measured using an Imes 950 spectrophotometer (Nireco, Japan). The former mode could determine Brix in whole tomatoes more accurately (Fig. 6). It appeared that the transmission mode was not good at determining quantity because the absorbance level was very high[2], while the interactance mode was superior to the transmission mode for measuring the internal properties of kiwifruit using VIS – NIR spectroscopy[28]. Moreover, there were some tomatoes for which the transmission spectrum could not be measured under the same conditions. When tomatoes of wide-ranging size were measured in transmission mode, the spectral measurement conditions had to be changed.

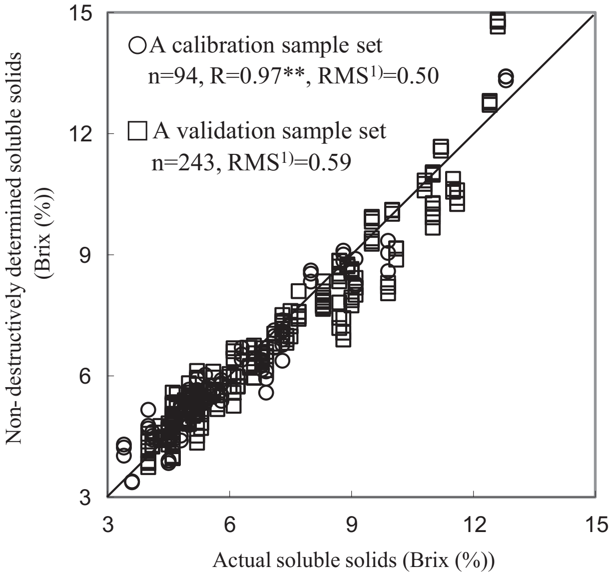


Fig. 5. Plots of actual and non-destructively determined soluble solids (Brix (%)) in whole tomatoes

The average of two non-destructively determined soluble solids values was plotted per tomato.

1) RMS(Root Mean Square)= $\sqrt{\frac{\Sigma (X-Y)^2}{n}}$

n=samples, X=actual Brix (%),
Y=non-destructively determined Brix (%)

3. Detecting internal disorder in intact vegetables

Vegetables with internal disorders are difficult to diagnose and consumers are unwilling to buy them.

Following optical measurement, the melon was cut vertically and the water-soaked symptoms within the area irradiated with an NIR beam were visually scored between 0 (sound) to 5 (the severest). The water-soaked symptoms occurred from the flesh below the blossom end. MLR analysis revealed a calibration equation using D^2 log 1/R at 810 and 942 nm. We tried to validate the MLR calibration using other melon sample lots; including not only own-cultivated but also purchased melons. Nevertheless, melons whose scores were predicted as exceeding 2.30 by the NIR method always showed the symptoms. The calibration was also able to detect browning flesh. A figure of around 810 nm was used to detect browning inside apples, while the water-soaked symptom in melons appears to be physiologically similar to browning in apples[23]. For practical use, an instrument (K-BA100, Kubota Corporation, Japan, (Figs. 2(b);

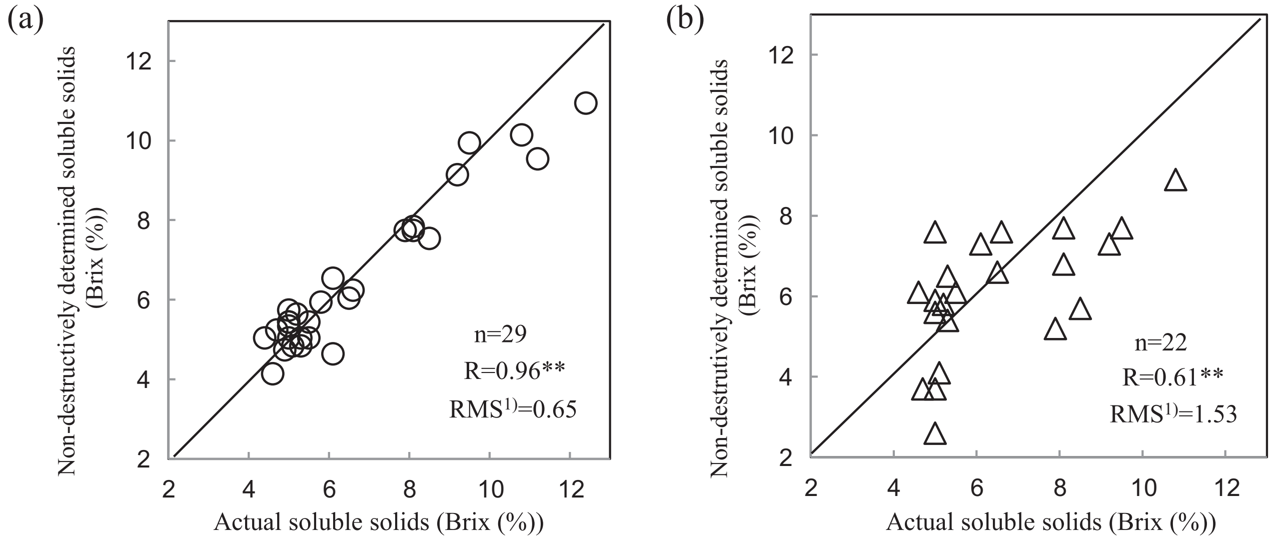


Fig. 6. Plots of actual and non-destructively determined soluble solids (Brix (%)) in whole tomatoes

(a) ‘Non-contact mode’ in interactance mode, (b) Transmission mode

1) RMS(Root Mean Square)= $\sqrt{\frac{\Sigma (X-Y)^2}{n}}$ n=samples, X=actual Brix (%),
Y=non-destructively determined Brix (%)

3(b))) was applied.

Browning of the inner scale in onions (Shingusare in Japanese) can also be detected using NIR spectroscopy (*In press*).

VIS-NIR spectroscopy

Many NIR spectrophotometers use a halogen lamp as a light source, in which case the NIR spectrophotometers can also obtain the VIS spectrum. VIS-NIR spectroscopy might include the potential to non-destructively determine lycopene in tomatoes, nitrate ions in vegetables and evaluate internal disorders in onions.

1. Lycopene in tomatoes

Pigments could have strong absorption in the VIS range, while VIS-NIR spectroscopy might have the potential to determine lycopene in tomatoes non-destructively when the absorbance of VIS range coincides with that of the NIR range.

As mentioned in lycopene above, lycopene content as a dependent variable was determined using the developed rapid determination method[11]. MLR analysis of spectra (500-1000 nm) revealed a calibration using D^2 log 1/R at 568, 602, 626, 692, 826, and 946 nm with MR of 0.97 (n =82). The MLR calibration was validated using other tomato sample lots, and the tomatoes were predicted well. Therefore, VIS-NIR technology was a potentially effective way to determine lycopene in tomatoes non-destructively[15]. Finally, D^2 log 1/R at 568, 626, 856 and 946 nm (Fig. 7) or 626, 856 and 946 nm were selected as independent variables of the MLR equation. The calibrations can be written respectively as follows:

Lycopene (mg/100g F.W.) in an intact tomato $= 11.66 - 257 \times D^2 \log 1/R_{568} + 7530 \times D^2 \log 1/R_{626} - 152000 \times D^2 \log 1/R_{856} - 18500 \times D^2 \log 1/R_{946}$

Lycopene (mg/100g F.W.) in an intact tomato $= 13.33 + 7680 \times D^2 \log 1/R_{626} - 171000 \times D^2 \log 1/R_{856} - 20000 \times D^2 \log 1/R_{946}$

2. Nitrate ions in intact vegetables

The nitrate-ion content of vegetables is a factor dictating food safety as mentioned in ISE above. However, no method for determining non-destructive nitrate ions in vegetables had yet been reported.

To measure the VIS-NIR absorption spectrum, each Japanese radish was manually placed on, or 3 mm away from the end of a fiber optic probe (the former is 'Contact interactance mode', the latter is 'Non-contact interactance mode'.) so that a Japanese radish was centered. The single correlation coefficients between d^2 log 1/R at 560 nm and nitrate-ion concentration were high (R=-0.86 for 'Non-contact interactance mode', -0.89 for 'Contact interactance mode, respectively). Subsequently, MLR analysis on the spectra (n=24) of 'Non-contact interactance mode' revealed a calibration using D^2 log 1/R at 560, 902, 864 and 904 nm with MR of 0.93. MLR analysis on the spectra of 'Contact interactance mode' revealed a calibration using D^2 log 1/R at 560, 902, 884 and 864 nm with MR of 0.93. When the results of the 'Non-contact interactance mode' and 'Contact interactance mode' were compared in validation sample sets, the former mode could determine nitrate-ion concentration more accurately[22] as non-destructive Brix determination in melons as mentioned above.

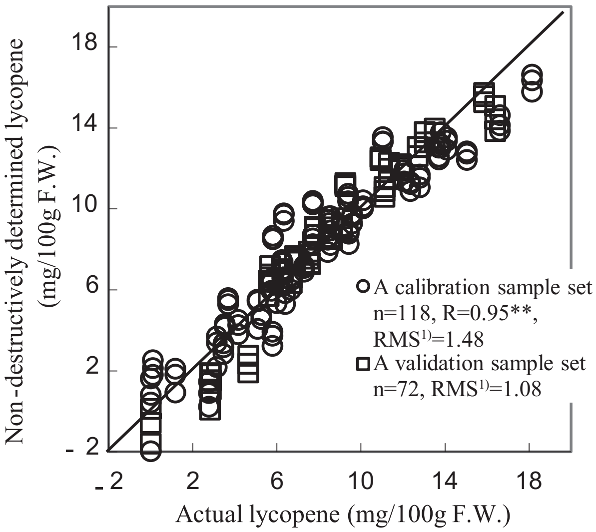


Fig. 7. Plots of actual and non-destructively determined lycopene content (mg/100g F.W.) in whole tomatoes

The average of two non-destructively determined soluble solids values was plotted per tomato.

1) RMS(Root Mean Square)= $\sqrt{\frac{\Sigma (X-Y)^2}{n}}$

n=samples, X=actual lycopene,
Y=non-destructively determined lycopene

The first target selected in leafy vegetables is the leaf stalk of Qing gin cai (*Brassica chinensis*) because it sometimes has high concentrations of nitrate ions. To measure the VIS-NIR absorption spectrum, each Qing gin cai was manually placed 3 mm away from the end of a fiber optic probe ('Non-contact interactance mode' (Fig. 2(b))) of an instrument (K-BA100, Kubota, Japan, (Fig. 3(b))) so that the leaf stalk was centered. D^2 log 1/R at 514, 902, 884 and 906 nm that relate to nitrate ions or carbohydrate were selected as independent MLR calibration variables. Subsequently, it emerged that 514 nm was identified as the wavelength proportionate to nitrate ions in aqueous potassium nitrate solutions[12], while the nitrate-ion concentration is related to carbohydrate concentration from a plant-physiological perspective. This VIS-NIR determination had a higher R than measurement using a handy colorimeter (NR-3000, Nippon Denshoku, Ind. Co., Ltd., Japan). However, Qing gin cais with very low nitrate-ion concentrations in winter were non-destructively determined less accurately though the sample temperature had negligible influence on the estimated values[14].

Vegetables of *Brassica* can be overwintered in Japan, whereupon the vegetables have low nitrate-ion concentrations. Conversely, lettuce not belonging to *Brassica* has a low nitrate-ion concentration. To elucidate the above-mentioned large error between actual and non-destructive nitrate-ion determination, attempts were made to non-destructively determine the nitrate-ion concentration of lettuces 'V' (Kaneko Seeds, Co., Ltd., Japan) grown in Nagano prefecture, Japan. Lettuces cannot overwinter in the area. To measure the VIS-NIR absorption spectrum of the stem using a K-BA100R spectrophotometer (Kubota Corporation, Japan, (Fig. 3(b))), each lettuce was manually placed 3 mm away from the end of a fiber optic probe ('Non-contact interactance mode' (Fig. 2(b))) so that the stem was centered. Though the equator of the lettuce has many measuring points, the stem end has only one. Log 1/R at 508, 546, 810, 890 and 906 nm were selected as independent MLR calibration variables (Fig. 8). The calibration can be written as follows:

Nitrate ions (ppm) in an intact lettuce stem end=$1830 - 11400 \times D \log 1/R_{508} + 14900 \times D \log 1/R_{546} - 170000 \times D \log 1/R_{810} + 859000 \times D \log 1/R_{890} - 691000 \times D \log 1/R_{906}$

508 and 906 nm related to nitrate ions and carbohydrate, respectively. It appears that overwintering has determined the non-destructive nitrate ions measurement less accurately.

3. Internal disorder in intact onions

The outer scale in onions is sometimes prone to browning, greening or decay (water-soaked symptoms) (Hadagusare in Japanese). The color may also have strong absorption in the VIS range. VIS-NIR spectroscopy may have the potential to determine non-destructively when the absorbance of the VIS range coincides with that of the NIR range.

To measure the absorption spectrum of an intact onion using a K-BA100R spectrophotometer (Kubota Corporation, Japan, (Fig. 3(b))), each onion was manually placed 3 mm away from the end of a fiber optic probe ('Non-contact interactance mode' (Fig. 2(b))) so that the upper side was centered. 2 spectra per onion were measured. Following optical measurement, the onion was cut vertically and the symptoms inside the area irradiated with a VIS-NIR beam were visually scored as 0 (sound), 0.5, or 1.0 (the severest). MLR analysis of spectra (750-1000nm, n=120) revealed a calibration equation using log 1/R at 750, 810 and 942 nm with MR of 0.86. We validated the MLR calibration using other onion sample lots (n=660). Nevertheless, 83.2% of onions in which the symptom occurred could be detected by the method[9].

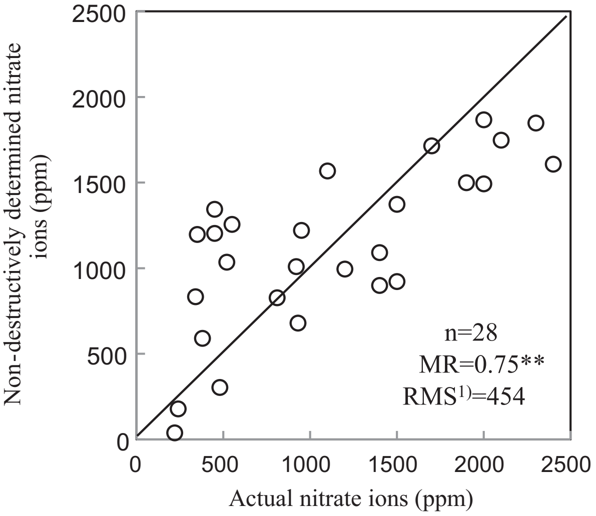


Fig. 8. Plots of actual and non-destructively determined nitrate ions (ppm) in the stem of intact lettuces

The actual nitrate ions in the stem end (2 cm length) were determined using a compact ISE (B-343, Horiba Ltd., Japan).

1) RMS(Root Mean Square)= $\sqrt{\frac{\Sigma (X-Y)^2}{n}}$

n=samples, X=actual nitrate ions,
Y=non-destructively determined nitrate ions

Independent calibration variables for non-contact interactance mode using NIR or VIS-NIR spectroscopy

As mentioned above, D^2 log 1/R in the vicinity of 902 and 880 nm were selected as key independent MLR calibration variables for Brix in fruit vegetables as onions[1]. D^2 log 1/R in the vicinity of 832 or 856 nm that converged at zero values[5] was selected as the third independent variable in the MLR calibrations. In addition, D^2 log 1/R at 856 was selected as the third independent variable in the MLR calibration for lycopene in tomatoes. The figure of 810 nm was used to detect browning inside apples and selected in the MLR calibrations for internal disorders in melons or onions. 568 and 626 nm were selected in the MLR calibrations for lycopene in tomatoes as first and second independent variables, respectively. Watada et al. (1976) selected the differences between absorbances at 570 and 780 nm to determine lycopene non-destructively[30]. D^2 log 1/R in the vicinity of 944 nm also converged at zero values, and was selected as an independent variable of MLR calibrations for internal disorders in melons, onions or the fourth independent variable of the MLR calibrations for lycopene in tomatoes. Around 511 nm related to nitrate ions was selected in the MLR calibrations for nitrate ions in stems of lettuce or leaf stalks of Qing gin cai as independent variables; combined with around 904 nm, one of the key wavelengths for carbohydrates[1]. The concentration of nitrate ions is related to carbohydrate concentration from a plant-physiological perspective.

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