How to get beautiful near infrared spectra

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First of all, I would like to express my sincere thanks to the ICNIRS and Buchi company for their generosity in presenting me this prestigious Tomas Hirschfeld Award for 2009. I feel such a great honour to be a part of this community, to work on NIR spectroscopy and to be the winner of the THA this year.

The title of my presentation is "How to get beautiful NIR spectra?" The term "beautiful" might not sound very scientific for many of the native English speakers who may prefer the term "informative." However, for me, as an agricultural engineer, a piece of successful research work and an invention are equal to producing an art-work. Therefore, when I look into the spectra, which are media for establishing a quality evaluation system, I examine and appreciate their beauty. Do they carry too much noise or too little information, a picture that many researchers might refer to as the Signal to Noise (S:N) ratio. I also look at the shape of the spectra to evaluate whether it is worthwhile for us to spend time doing any possibly unrewarding calculations or not.

It is true that chemometrics is a powerful tool to extract useful and interesting information; but if there is no clear relationship between the spectral signal, and the things that are to be measured it would be nearly impossible for even the best chemometrics to squeeze something out. Over the years, I have found that to design an appropriate sample presentation, and use reference materials having optical density compatible with the samples-to-be are the key toward achieving "Beautiful spectra."

During 1987–1993, our mission at the National Food Research Institute (NFRI) was to develop an evaluating system for fruit sweetness using NIR spectroscopy. The technology has become a standard in the Japanese fruit market nowadays and is on high demand in the international market. Our first experiments concerned peaches, an easy fruit to work with, with quite thin peel.[1] Following the work of Birth and Dull for onion and cantaloupe,[2,3] I used the Interactance Probe commercially available from the Pacific Scientific Company (now Foss Analytical). Due to the round shape and the size of the fruit that was bigger than the probe header, we developed a fruit holder to hold the fruit steadily, and to prevent stray light. We then obtained a successful calibration equation with a very simple MLR equation, using information from amorphous glucose (Figure 1).

The next target was Satsuma orange, a fruit with thicker peel, with a white mesocarp underneath the peel. With this fruit the interactance mode could not provide good results, because the reflected light from the peel and the white part was too strong. To solve that, we used the transmittance mode, with a silicon detector mounted underneath the sample to maximise the signal from flesh [Figure 2(a)].[4]

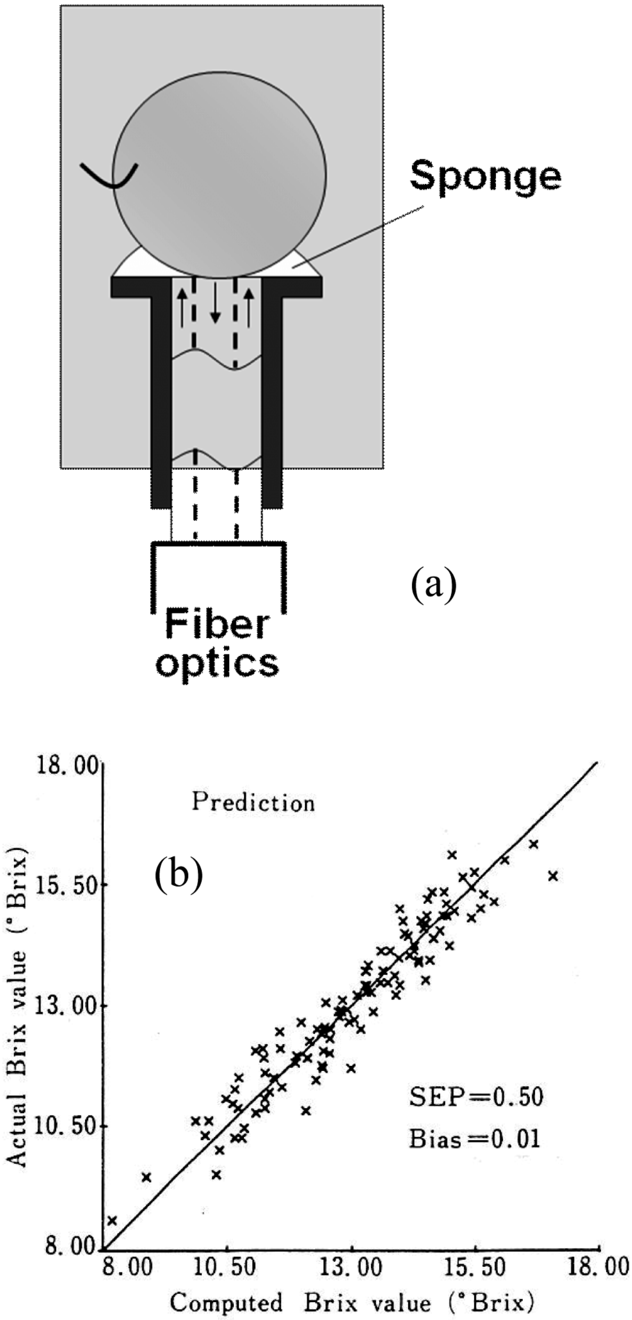

Figure 1. Sample presentation for intact peaches in the interactance mode (a) and (b) the validation results for Brix value calculated from four-wavelength MLR calibration equation.[1]

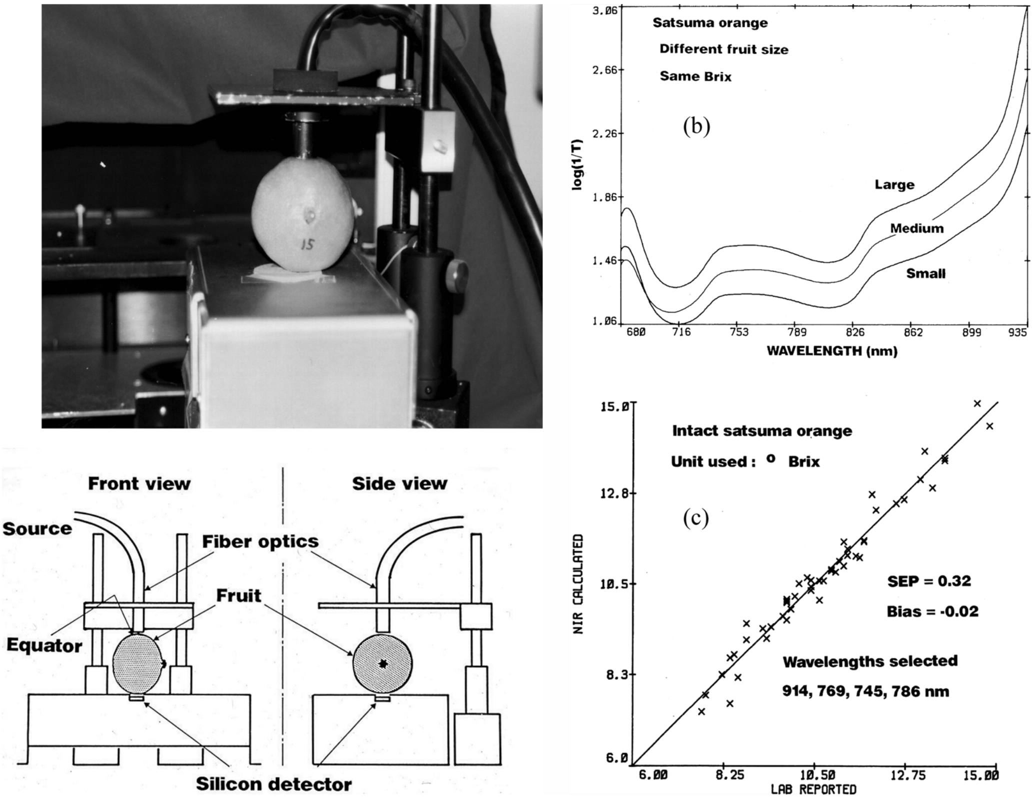

Figure 2. (a) Sample presentation of intact Satsuma in the transmittance model. (b). Sample size effect on the original spectra and (c) the validation result for Brix values calculated from four-wavelength MLR calibration equation.[4]

After removing the sample size effect using water information, impressive results for Brix prediction could be obtained, with values of $R^2=0.98$ and $SEP=0.32°$ [Figures 2(b,) and (c)]. Later, in 1995, we found that sample temperature could affect the state of water in the sample to a great extent; leading to variable errors in calibration.[5] We could solve the problem at that time by using a temperature compensation equation, a technique that has been proved by various researchers as a very effective method. However, two years ago I considered that we might be able to work on the spectra before making calculation, not by removing temperature information, but adding temperature fluctuation into only one sample, to simulate a set of samples with different temperatures.[6] The good news is that we achieved success and the method might be an improvement on the conventional method, whereby a number of spectral acquisitions on a sample set with different temperatures is needed.

Another work on fruit that I would like to mention is the on-tree NIR measurement to evaluate the maturity level of mangoes prior to harvesting. To get beautiful spectra, various studies had been conducted including the effect of sunlight, temperature and the use of a portable instrument in the very early part of the day. To provide a stable power supply we avoided the use of a built-in battery, which was quite heavy and could not last for the several hours needed at that time, but connected the power supply cable that was 30-m long to the cigarette lighter socket of a car. Nowadays, many workers are using either portable instruments or imaging systems, as you have seen, and will see in this Conference. We are very proud to have been one of the pioneers for the use of portable NIR instruments. Figure 3 demonstrates the effect of sunlight on NIR spectra measured outdoors, and the method used to correct these effects.[7]

Beside the NIR measurement of fruits, our laboratory has also developed various kinds of sample cells and sample holders, such as the cells for single kernels of grains,[8-10] and test tube holders for blood collection tubes, and common test tubes that can be used to replace quartz cuvettes.[10-14] Furthermore, during these years, we found that the selection of reference materials for NIR measurement to comply with the optical density of the sample to be scanned would improve the accuracy of calibration equations to a great extent. Figure 4 shows the NIR spectra of mixed vegetable juice kept in a test tube, measured against four kinds of reference materials: an empty test tube (Air), 1-mm thick ceramic, 1-mm thick Teflon and 2-mm thick Teflon.

The signal of transmitted light measured from the sample was 2.436 Volts, while those of Air, 1-mm ceramic, 1-mm Teflon and 2-mm Teflon were 5.5140, 1.624, 3.956 and 2.669 Voltage, respectively. Based on our experience, instead of using an internal reference material supplied by instrument manufacturers, the use of an appropriate custom-made reference material would

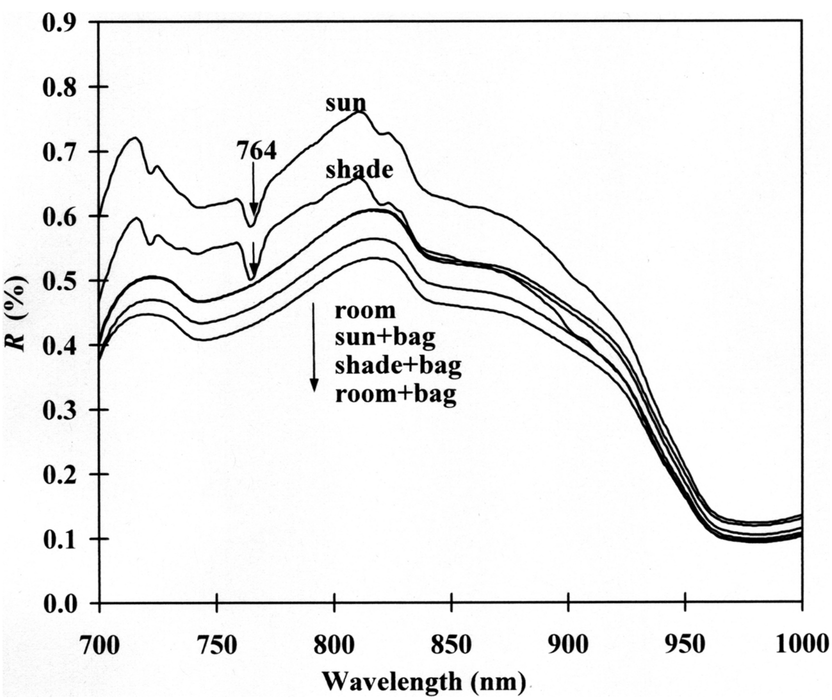

Figure 3. Effect of sunlight on the reflectance spectra of mango fruit measured outdoor.[7]

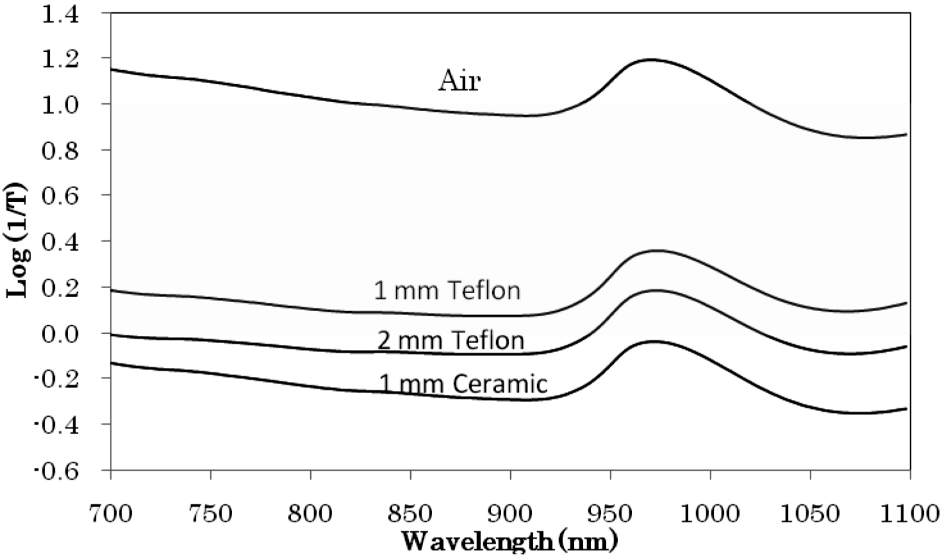

Figure 4. NIR spectra of mixed vegetable juice kept in a test tube measured against different types of reference material.

provide better quality NIR spectra, with the range of 0 to 1 absorbance units. The figure indicates that the 1-mm Teflon reference was suitable for NIR measurement of this type of material.

My conclusion here would be to concentrate on obtaining meaningful and consistent ("beautiful") spectra before attempting to perform either conventional or sophisticated calibrations. Excellent spectra can best be obtained through the use of an appropriate (possibly custom-designed) sample presentation system, together with reference materials that are in accordance with the samples. The next step is to determine and evaluate other factors that can affect the spectra, and solve them one-by-one. Then assemble a set of samples that include all of the likely variance. Finally, do not forget to determine the error of the wet chemistry, and understand both your samples and your constituents.

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