Opening the black box of NIR prediction for sugar content of fruits: a complementary analysis by aquaphotomics and metabolomics

Akifumi Ikehata

Food Research Institute, National Agriculture and Food Research Organization (NARO),
Tsukuba, Japan,
e-mail: ikehata@affrc.go.jp

Nondestructive measurement of sugar content (Brix or soluble solids content) of fruit is known as a widely-spread near-infrared (NIR) application. However, the mechanism of the prediction model is not necessarily understood (black box) because of the deficient band assignment. To reveal the molecules contributing the NIR prediction model, we are working with NMR based metabolomics. Metabolomics is an authorized comprehensive study of metabolites (small organic molecules) based on quantitative measurement of the dynamic multiparametric metabolic response of living systems. For this purpose, chromatography-mass spectrometry and NMR spectroscopy are used as the primary techniques. High separation performance and well assigned spectra of these techniques are helpful to understand the contribution of metabolites in the NIR prediction model. However, it is also known that some important NIR wavelengths are embedded in absorption bands of water and they are not assigned to absorptions of any metabolites. This fact implies the necessity of a complementary analysis from viewpoints of both metabolomics and effect of water (i.e. hydration and hydrogen bonding), that is aquaphotomics, for understanding the mechanism of NIR applications to various biosystems, agricultural products and foods. In this paper, we demonstrate the way to open the black box with the aid of proton nuclear magnetic resonance (^{1}H-NMR).

VIS-NIR spectra (500-1000 nm) of apples and peaches in wide ranges of sugar content were measured by a portable spectrometer (Kubota, K-BA100R) equipped with an intaractance probe. Extracted juice from the flesh of the measurement site of VIS-NIR spectra was diluted with deuterium phosphate buffer to set the pH 7.0 and measured by an NMR instrument (Bruker, AVANCE 500 MHz). The given NIR and ^{1}H-NMR spectra were analyzed by heterospectroscopy (SHY) method, which calculates correlation coefficients at all combinations of variable channels i.e. wavelength and chemical shift. The heatmap of SHY showed us obvious positive or negative correlations between ^{1}H-NMR signals of sugars and the primary components at 830, 888 and 904 nm of NIR spectra, which are frequently used for practical multiple regression models. However, the latter two wavelengths may not be directly caused by sugars. The result implies a possibility that water interacting with solutes plays an important role in the prediction model for sugar content of fruits. Water molecules form hydrogen bond network in different ways depending on what they are interacting with. To compare with the NIR spectra fruits, model solutions of sugars and organic acids were also examined. Other meaningful correlations were also observed in the SHY heatmap, for example, the prediction model for peach seems to be formed not only by sugars, but also by hydrolysis of pectin; a decomposition reaction with water.

Although NIR prediction for biosystem is easily given by a regression software, it involves various metabolomes and water in the background. Therefore, we emphasize the importance of complementary analysis by aquaphotomics and metabolomics for further development of spectroscopic techniques.

Keywords: ^{1}H-NMR, heterospectroscopy, band assignment of NIR spectra, apple, peach