Nondestructive quality evaluation and monitoring of Braeburn apples by Spatially Resolved Spectroscopy

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ABSTRACT

Contact Spatially Resolved Spectroscopy (SRS) measurements by means of a fiber-optics probe were employed for nondestructive assessment and monitoring of Braeburn apples during shelflife storage. SRS measurements and estimation of optical properties were calibrated and validated by means of liquid optical phantoms with known optical properties and a metamodeling method. The acquired optical properties (absorption and reduced scattering coefficients) for the apples during shelf-life storage were found to provide useful information for nondestructive evaluation of apple quality attributes (firmness and SSC) and for monitoring the changes in their microstructure and chemical composition. On-line SRS measurement was achieved by mounting the SRS probe over a conveyor system.

1 Introduction

To date, conventional Visible and Near Infrared (Vis/NIR) spectroscopy has been successfully employed on laboratory scale for nondestructive evaluation of food quality (Nicolaï et al., 2007; Huang et al., 2008; Aernouts et al., 2011). As light interacts with a food sample its spectrum is changed due to scattering by the sample microstructure and absorption by chemical components. This allows to predict food quality attributes from the observed changes in the light spectrum by using multivariate calibration methods (Martens & Næs, 1991). Since the basic background of most of these calibration models is based on Beer's law, the effects of multiple scattering increasing the path length of the light in the sample have to be reduced or minimized to improve the prediction accuracy during model construction (Nicolaï et al., 2007). However, this multiple scattering phenomenon also carries information about the sample microstructure which, in most of the cases for foods, could be very valuable for predicting many important macrostructural food quality attributes such as the texture or crispness. Therefore, separating the information on light scattering and absorption in the studied food samples would allow to assess both the textural and composition properties simultaneously, which is not possible with conventional NIR spectroscopy techniques (Nicolaï et al., 2007).

Spatially resolved spectroscopy (SRS) has been successfully used in biomedical research for noninvasive medical diagnoses (Tuchin, 2007). The basic principles of these diagnoses are that the progress or status of the existing disorders in tissues/organs can be detected with minimal invasion through changes in their absorption or scattering properties. This study, therefore, inspired by these achievements of SRS in the biomedical domain, aimed at adapting this technology for food applications. By implementing multiple conventional Vis/NIR spectroscopy measurements on the food samples at different spatial locations, scattering and absorption could be separated from the post-interacting lights by light propagation models (Nguyen Do Trong et al., 2011; Herremans et al., 2013) or metamodels (Nguyen Do Trong et al., 2013). These scattering and absorption levels, represented by optical properties (absorption coefficients, scattering or reduced scattering coefficients) will then be related to the food quality attributes for nondestructive food quality assessment.

This research, therefore, aimed at investigating the potential of monitoring the changes in microstructure and chemical composition of the Braeburn apples by means of their absorption and reduced scattering coefficients introduced by 2 weeks of shelf-life storage, and at constructing calibration models employing these acquired optical properties to predict the apple quality attributes (soluble solids contents (SSC) and firmness).

2 Material and Methods

2.1 Contact SRS setup

Setup description

The SRS setup used in this research is the same as the one used by Nguyen Do Trong et al. (2011) and Herremans et al. (2013). In brief, it contains one illumination fiber and five detection fibers collecting diffuse reflected lights in the range 400 – 1000 nm. The fibers used were multimode fibers (Thorlabs, USA) with a numerical aperture of 0.22 and a core diameter of 200 μm. All these fibers were integrated in a fiber-optics probe for contact SRS measurements. In the probe, the detection fibers were accurately placed at center-to-center distances ranging from 0.3 to 1.2 mm from the illumination fiber. The illumination fiber delivered the light from a halogen light source (AvaLight-DHc, Avantes, The Netherlands) to the measured samples. The detection fibers guided the diffuse reflected photons from the samples to a spectrograph (Horiba Jobin-Yvon, New Jersey, USA) splitting these into many waveband components which are projected onto a CCD camera (Hamamatsu, Louvain-La-Neuve, Belgium). Signals from the camera were acquired by means of a data acquisition card and a custom written LabView program (National Instruments, USA). In Fig. 1 the SRS setup is schematically illustrated.

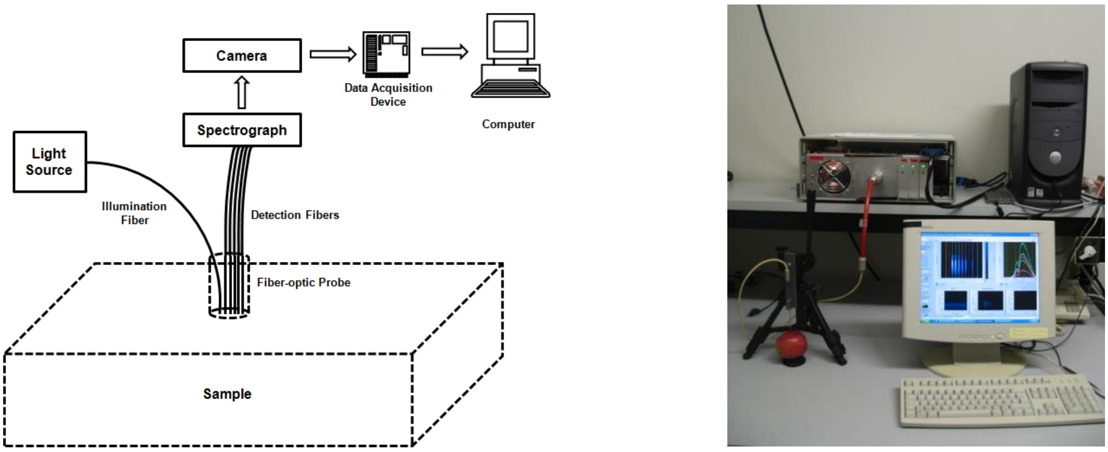

Fig. 1 (Left) Schematic illustration of the SRS setup. (Right) SRS measurements in action on an apple.

Setup calibration and measurement

Wavelength calibration was carried out to link each pixel on the spectral dimension of the CCD sensors to the corresponding wavelength band by means of a calibration light source (AvaLight-CAL-2000, Avantes, The Netherlands). The plot of the acquired wavelength versus pixel number showed a very good linear relation ($R^2 = 0.98$) (data not shown). Different efficiencies of the detection fibers were compensated by positioning the probe inside a 50 mm diameter integrating sphere (Avantes, The Netherlands) illuminated from its side port and reflecting more than 98% of the light intensity in the range 400-1000 nm to collect the homogeneously diffuse reflected lights from the sphere wall.

The relative diffuse reflectance spectra of the detection fibers from a measured sample were computed as the ratios of the dark-corrected intensities acquired for that sample by the dark-corrected intensity collected in the integrating sphere. In this way, the measured signals were compensated for dark noises, variations in light source intensity, differences in efficiencies of different pixels of the camera and differences in efficiencies of the detection fibers. Each contact SRS measurement provides 3D data containing various wavelength-dependent spatially resolved diffuse reflectance profiles of the studied samples.

2.2 Braeburn apples

Thirty Braeburn apples were harvested at the experimental orchard of KU Leuven, Belgium following normal cultivation condition and were transported to the lab. In the lab, these apples were measured with the SRS setup (day 0). On each apple, SRS measurements were carried out at 7 different positions on the equator at each side of an apple (most reddish and most greenish sides) and were then averaged to acquire the measured data for that side. Half of the apples (noted as Group A) went for destructive measurements (soluble solids contents (SSC) and firmness) at the same areas previously analyzed by SRS and the other half (noted as Group B) were then stored under shelf-life condition (18 ^{o}C in a controlled temperature storage cell) for 2 weeks. After the shelf-life storage of 2 weeks, SRS and destructive measurement procedures were implemented again (day 14) for these fifteen apples at the same regions as in day 0.

Firmness of the apples was measured by means of a universal testing machine (LRX, Lloyd Instruments) and was recorded as the maximum force needed to penetrate the sample 8 mm with a cylindrical plunger (diameter 1.13 cm). The juice coming out during plunging was quickly extracted; its SSC was analyzed by a digital refractometer (αAtago PR-101α, Atago Co. Ltd., Japan) and was recorded in ^{o}Brix.

2.3 Estimation of optical properties of the apples

Thirty six optical liquid phantoms containing aqueous mixtures of intralipid 20% (Fresenius Kabi, Sweden) serving as scatterers and Indian ink (Chartpak Inc., USA) acting as absorber in the range 400 – 1000 nm were prepared. Optical properties of these phantoms were determined as reported in Nguyen Do Trong et al. (2011) and covered wide ranges of optical properties for food and agricultural products. A metamodeling method (Nguyen Do Trong et al., 2013) was then developed and applied to the measured SRS data of the phantoms to relate them to the reference optical properties. To validate this metamodeling method, one phantom (validation phantom) was left out of the phantom set and the remaining 35 phantoms were used for constructing the calibration metamodels. These acquired calibration metamodels were then used in inverse estimation to estimate the optical properties of a new spatially resolved diffuse reflectance profile by searching for the best optical properties in the calibration data which would provide the best fitted profile to the input profile. Comparing the estimated optical properties of this validation phantom from the inverse estimation with its reference optical properties allowed to calculated the accuracy of the inverse estimation procedure over the range of optical properties covered by validation phantom sets. In this research, 8 phantoms covering the range of optical properties relevant for apples were selected and each of these was used in the 8 validation steps. Global calibration metamodels were then constructed from all of the 36 liquid phantoms and were applied to the measured SRS data of the apples to estimate their optical properties.

3 Results

3.1 SRS measurements of the apples

The relative diffuse reflectance spectra acquired at the different fiber positions acquired for a Braeburn apple are shown in Fig. 2. The wavelength range has been reduced to the range with high signal-to-noise ratio.

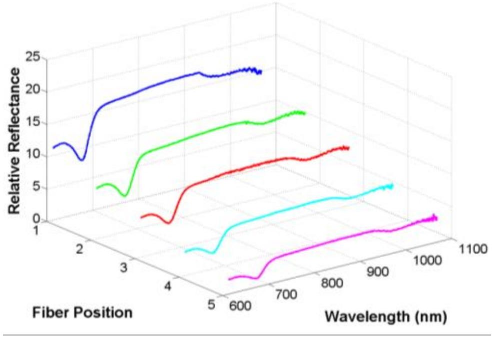

Fig. 2 Spatially resolved diffuse reflectance spectra of a Braeburn apple

In the diffuse reflectance spectra obtained with the different detection fibers two ‘valleys’ can be observed which can be related to the absorption peaks of chlorophyll (at 670 nm) and water (at 970 nm). At each wavelength, the diffuse reflectance decreases with increasing fiber number or source-detector distance because the lights have to travel a longer path through the sample to reach this fiber and thus have had more chance to be scattered or absorbed.

3.2 Measurement validation on optical phantoms

In Fig. 3 the predicted optical properties of the 8 validation phantoms are plotted against their reference optical properties.

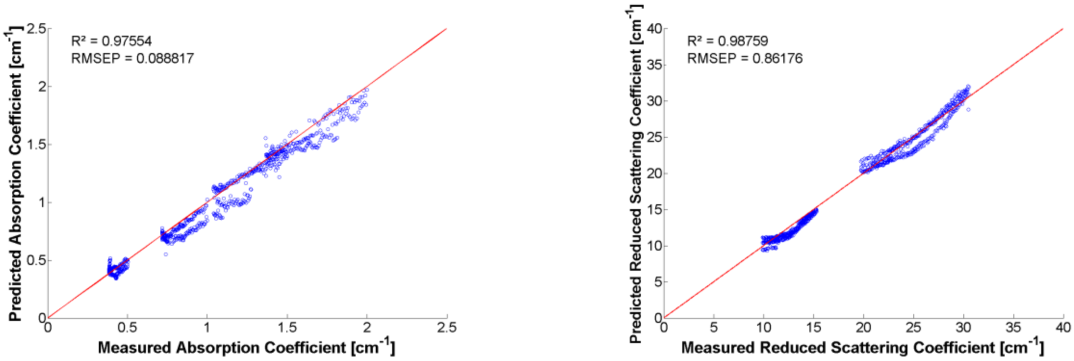

Fig. 3 Plots of predicted against measured optical properties of the 8 validation phantoms: (left): absorption coefficients; (right): reduced scattering coefficients. Four outliers were removed (< 0.5%). Diagonal red line indicates perfect prediction line. *RMSEP*: Root Mean Squared Errors of Prediction.

It can be seen that the prediction performance of the inverse metamodel for the estimation of the absorption and reduced scattering coefficients of the validation phantoms is very good (R^2-absorption coefficients = 0.975 and R^2-reduced scattering coefficients = 0.987). Moreover, the mean prediction errors (*RMSEP*) are approximately 0.089 cm^{-1} for the absorption coefficient and 0.862 cm^{-1} for the reduced scattering coefficient. The fact that these errors are small compared to the absolute values of the optical properties used in the validation procedure supports the usefulness of the metamodeling method for estimating the optical properties of food samples such as apples from the acquired SRS measurement data.

3.3 Optical properties of the apples

The mean optical properties (absorption and reduced scattering coefficients) of the group A and B, each of which contained 15 apples, are plotted in Fig. 4.

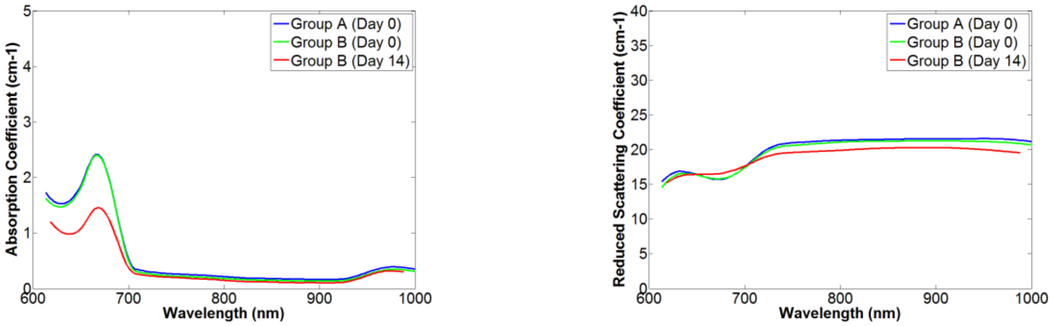

Fig. 4 Mean values of the estimated optical properties of the Braeburn apples: absorption coefficient (left) and reduced scattering coefficient (right).

In Fig. 4 (left), the apple absorption coefficient spectra clearly show two absorption peaks: one at 670 nm, representing the presence of chlorophyll, and another at 970 nm, corresponding to water in the apple tissues. From 700 to 900 nm, the absorption coefficients are small. These observations are in good agreement with the fact that chlorophyll and water are the two main chemical constituents which absorb most strongly in the range 600 – 1000 nm. The chlorophyll peak of group B at day 14 is lower than that at day 0; which clearly indicates the degradation of chlorophyll during ripening of the apples under shelflife condition. Interestingly, the absorption peak for chlorophyll is much higher than for water, which supports that the fiber-optics probe mostly captured diffusely reflected lights penetrating the regions within the skin or down to the flesh, but still close to the apple skin because of the short source-detector distances. In these regions, chlorophyll is more dominant than water. The absorption spectra of group A and B (day 0) are very similar, which could be because these apples are in the same maturity condition (at the beginning of shelflife storage).

In Fig. 4 (right), the reduced scattering coefficient spectra of group A and B (day 0) are close and higher than those of group B (day 14). This strongly reflects the changes in microstructure of the apples during shelf-life storage. Since light scattering mainly happens at the boundary of two media in the apple tissues with different refractive indices (e.g. between cell walls and their surroundings, internal cell media and cell compartments, etc.), the scattering reduction here clearly indicates the degradation of cell wall and other compartments, water loss, etc. inside the apples during storage. The scattering profiles in the range 750 – 1000 nm are quite flat, which could be explained, according to Mie theory for light scattering, by the wide distribution of the scatterer sizes within the apple tissues: the cells and their compartments. The valleys appearing in the scattering spectra of the 3 groups around 670 nm could be explained as the inability of the inverse metamodeling method to completely separate the absorption and scattering phenomena in the wavelength regions where the absorption is relatively high compared to the scattering.

3.4 Evaluation of apple quality (Firmness and SSC)

The estimated optical properties of the Braeburn apples in group A (day 0) and B (day 14) were used to relate to their corresponding quality attributes (Firmness and SSC) by means of Partial Least Squares (PLS) models (PLS Toolbox 5.0, Eigenvector Research, Inc., USA). Optical properties in the regions below 650 nm and above 980 nm were excluded because the signal to noise ratio was too low in these regions. To select calibration and validation samples for the PLS models, firmness and SSC were first sorted in descending order. The optical properties data were also rearranged according to these corresponding orders of firmness and SSC. From the top samples with highest firmness or SSC, the first two were selected for the calibration set and the third was included in the validation set; and so on. This resulted in a calibration set containing 40 samples (two thirds of the original data set) and a validation set with 20 samples (one third of the original data set). The Leave One Out Cross Validation method was used to select the best calibration models. The plots of predicted against measured firmness and SSC of all the samples are illustrated in Fig. 5.

The firmness and SSC are moderately predicted based on the absorption coefficient spectra (top row) (r (Pred, SSC) = 0.657; RMSEP (SSC) = 0.665 (^{o}Brix) and r (Pred, Firmness) = 0.766; RMSEP (Firmness) = 1.165 (kgf)). The prediction performance based on the reduced scattering coefficient spectra is slightly worse for firmness (r (Pred) = 0.613) and similar for SSC (r (Pred) = 0.543) (bottom row). The fact that the prediction of SSC based on the absorption coefficient spectra is far better than that based on the reduced scattering coefficient spectra could be attributed to the more direct correlation between the chemical composition (SSC) of the apples and their absorption properties than between the microstructure of the apple tissues represented in scattering properties and their chemical composition. On the other hand and surprisingly enough, predicting firmness based on the absorption coefficient spectra is considerably better than that based on the reduced scattering coefficient spectra whilst the opposite would be expected. This interesting outcome could be explained by the fact that the apple tissues analyzed by the probe were shallow and close to the apple skin while the firmness measurement by the universal testing machine probes the flesh much deeper.

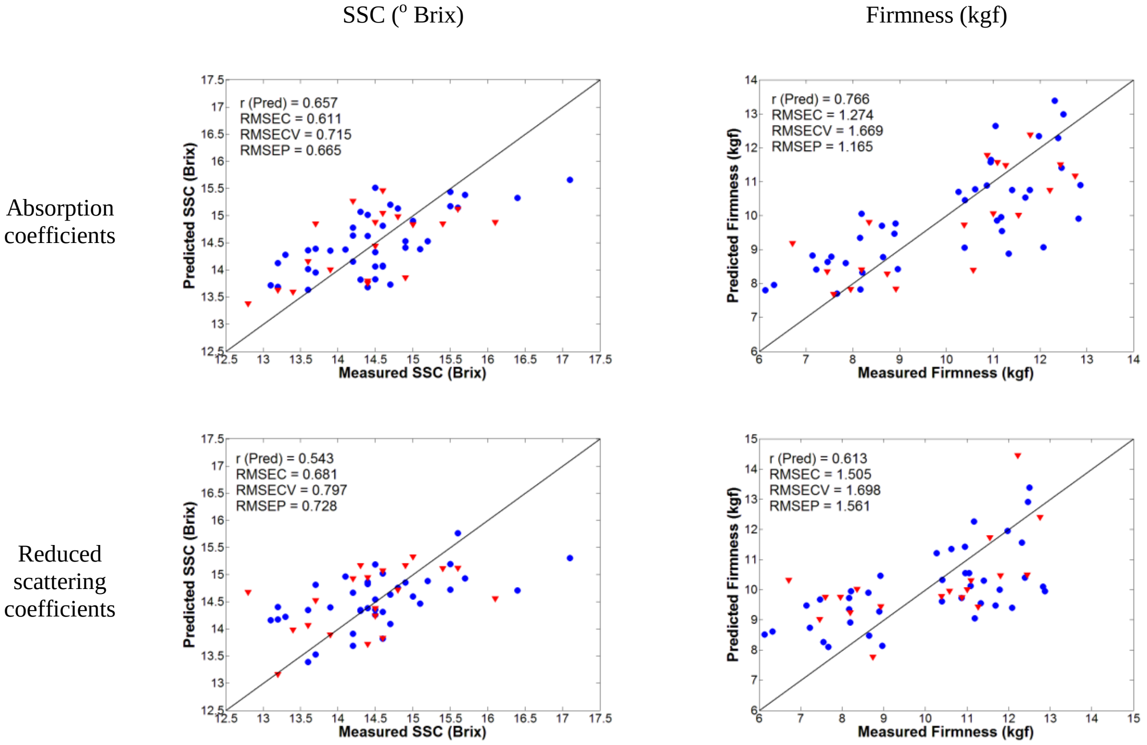

Fig. 5 Prediction of SSC and Firmness based on the absorption and reduced scattering coefficient spectra with PLS regression: Diagonal black lines represent perfect prediction lines. *RMSEC*: Root Mean Squared Errors of Calibration; *RMSECV*: Root Mean Squared Errors of Cross Validation; *RMSEP*: Root Mean Squared Errors of Prediction; Blue circles: Cross Validation samples; Red triangles: Validation samples.

4 Discussion

The metamodeling method employed in this research for estimating optical properties showed many of its advantages: fast, sufficiently accurate and potential for on-line applications. The apple optical properties (absorption and reduced scattering coefficients) obtained in this research by means of contact SRS measurements proved to be promising parameters for monitoring changes in the microstructure and chemical composition of the Braeburn apples during shelf-life storage. They also have potential for nondestructive assessment of apple quality attributes (firmness and SSC).

To investigate the possibilities of implementing on-line contact SRS measurements, the contact SRS setup in this research was also integrated onto an industry-mimicking conveyor controlled by a PLC controller system at Universidad Politécnica de Madrid, Spain and was tested for its on-line performance. The outcomes were very promising: successful functioning with sufficiently fast measuring speed, and good repeatability (proofs not shown). However, regarding to operations in the food industry, a hyperspectral or multispectral camera would be more preferable to perform on-line noncontact SRS measurements for solid foods like apples. For liquid, semi-liquid or paste foods, on-line contact SRS measurements by a fiber-optics probe with proper procedures to assure hygienic requirements would be a good choice.

5 Conclusions

A setup for contact SRS measurements by means of a fiber-optics probe was successfully developed and validated in the lab by means of liquid optical phantoms with known optical properties and a metamodeling method. The estimated optical properties for Braeburn apples measured by this setup during shelf-life storage were found to be promising parameters for nondestructive evaluation of apple quality attributes (firmness and SSC) and to have potential for monitoring the changes in their microstructure and chemical composition. The possibilities for implementing on-line SRS measurements were also confirmed.

Acknowledgements

This publication has been produced with the financial support of the European Union (project FP7-226783 - InsideFood). The opinions expressed in this document do by no means reflect the official opinion of the European Union or its representatives. The aid from Professor Depeursinge and his co-workers at EPFL, Lausanne, Switzerland are greatly acknowledged.

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