Non-destructive Characterization of Food Microstructure and Composition by Spatially-Resolved Spectroscopy


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ABSTRACT

Food quality is critically determined by its microstructure and composition. These properties could be quantified non-invasively by means of optical properties (absorption and scattering coefficients) of the food samples. In this research, a spatially-resolved spectroscopy setup based on fiber-optic probe was developed for acquiring spatially-resolved reflectance profiles of optical phantoms and microstructured foods (model foods) in the range 400 – 1100 nm. A model for light propagation in turbid media based on diffusion approximation for solving the radiative transport equation was employed to derive optical properties of these phantoms and model foods. Results of solid phantoms indicated that diffusion equation is sufficiently accurate for modeling light propagation in the investigated samples. Derived reduced scattering coefficients \( \mu'_s \) of the model foods obviously showed a logical correlation with the corresponding microstructure analyzed by optical microscopy. Estimated absorption coefficients \( \mu_a \) were also in good agreement with the designed ingredients. The research results clearly support the potential of spatially-resolved spectroscopy for non-destructive food quality inspection and process monitoring in the food industry.

Keywords: spatially-resolved spectroscopy; light scattering and absorption; diffusion equation

INTRODUCTION

Quality of foods strongly depends on their microstructure and composition. Examples include sponginess of bread, crispness or crunchiness of crackers, firmness or sweetness of fruits. Processing of foods also affects their microstructure and composition: existing structures are destroyed and new ones are formed; some constituents are changed and new ones are created. Therefore, rapid and accurate measurement of food microstructure and composition and how they change during processing operations is essential for the production of high quality foods.

Microstructure and composition (and their changes) strongly determine light propagation behavior (e.g. diffuse reflectance) in the illuminated food samples, mostly attributed by scattering and absorption phenomena [1]. Light absorption in food matrices is molecule-specific and theoretically described by Beer's law. The scattering is, on the other hand, characterized by changes in trajectories during propagating of electromagnetic waves (light) induced by their interactions with food structures like cell wall, nuclei, membrane, mitochondria, particles, air pores,... whose size is in the same order of magnitude as the photon wavelengths (0.4-2.5 \( \mu \)m) and by differences in refractive indices at these microstructure interfaces. Because of the fact that food quality attributes like firmness and sweetness in fruits, tenderness in meats, crispness or crunchiness of crackers... are mostly determined by chemical composition and microstructure of the food samples, these qualities could be evaluated non-destructively if the levels of light absorption and scattering in food matrices are quantified. However, multiple light scattering increases photon pathlengths inside the biological structure resulting in increased absorbance for the same concentration level, such that the measured reflectance or transmittance spectra result from the interplay of both scattering and absorption. To date, traditional Vis/NIR spectroscopy has been successfully employed for non-destructive, on-line quality inspection of foods and agricultural products [2, 3]. Since this traditional technique only measures the diffuse reflectance (or transmission), which is a combination of scattering and absorption effects, further improvements could be implemented for resolving the aforementioned scattering-absorption interaction effects in the measured signals prior to further analyses for a better quality prediction performance. Several data pre-treatment methods have been developed for reducing scattering effects such as Multiplicative Scatter Correction (MSC), Extended Multiplicative Signal Correction (EMSC), etc. [4, 5]. However, variations in the microstructure still often lead to deviations in the predicted values and require recalibration. Accurate separation of the information on scattering and absorption contained in the acquired spectra would thus promote more accurate non-destructive quality inspection of foods during storage or process monitoring. One way to obtain model-based separation of the scattering and absorption properties of a samples non-destructively is by combination of multiple spectroscopic measurements on the same sample at different
distances from the illumination source (spatially-resolved spectroscopy). Several researchers have used this approach to investigate the correlation between scattering and microstructure-driven properties of meats [6, 7] or to construct optical-property-based calibration models for prediction of macroscopic properties of fruits [8, 9]. Although correlations between reduced scattering coefficients and these macroscopic properties have been found, large variations in microstructure and composition within one sample and among different samples often lead to broad ranges of acquired reduced scattering coefficients, as observed for apple skin and flesh of different cultivars [10]. Model systems with different designed microstructures simulating different and desirable food structures would be very helpful to get better insight in the light propagation in real matrices of foods and agricultural products.

In this study, the potential of spatially resolved spectroscopy for non-invasively characterizing microstructure and composition of the microstructured foods (model foods) by means of their optical properties (absorption and reduced scattering coefficients) has therefore been investigated.

MATERIALS & METHODS

Spatially-Resolved Spectroscopy Setup (SRS Setup)

A setup for SRS measurements in the 400-1100 nm range has been built. This setup consists of a contact probe with accurately placed fibers which is linked to a spectrograph for simultaneous measurement of the reflectance at the different distances by a CCD camera. The optical probe has been designed and assembled at the Swiss Federal Institute of Technology (EPFL, Lausanne, Switzerland). The fibers used are Thorlabs multimode silica fibers (FVP-200 PF) with a numerical aperture of 0.22 and a core diameter of 200 μm. The 7 detection fibers are placed at various distances from the illumination fiber, ranging approximately from 0.3 to 1.2 mm with a step of about 0.15 mm. The illumination fiber of the probe is connected to a AvaLight-DHc (Avantes, Eerbeek, The Netherlands) halogen lamp through an optical switch. The detection fibers from the SRS probe and a fiber from the optical switch of the light source are aligned in the entrance slit of a CP200 133 g/mm spectrograph (Horiba Jobin-Yvon, New Jersey, USA) which splits the light from each of these fibers into its spectral components and projects these onto a Hamamatsu C7041 CCD camera with a S7041-1008 detector (Hamamatsu, Louvain-La-Neuve, Belgium). The signal from this camera is transferred to a computer by means of a PCI MIO-16E-4 data acquisition card. Control of the light source, optical switch and camera is performed in LabView software (National instruments, TX, USA). The measurement setup for spatially resolved spectroscopy is illustrated in Figure 1.

Optical characterization procedure

A typical solution of the radiative transport equation for light propagation in turbid media based on diffusion approximation presented in [11] was used to fit the spatially-resolved reflectance profiles of a sample acquired at the different wavelengths by the SRS setup by means of a trust-region non-linear least squares fitting algorithm for estimation of the optical properties: absorption and reduced scattering coefficients. All the fittings were implemented in Matlab (version 7.5, The MathWorks Inc., Natick, USA). The accuracy of the fitting procedure was evaluated by implementing the same procedure on 16 solid phantoms with known optical properties prepared by the same method as presented in [12]. In short, these solid phantoms contain TiO₂ powder serving as scatterer and black toner ink acting as absorber in an epoxy resin medium.
Preparation of the model foods

Since the aim of this research is to evaluate the potential of spatially-resolved spectroscopy to separate the information on the microstructure from that on the chemical composition and to get insight in the propagation of light through foods with different microstructures, several model foods with clearly different microstructure properties have been designed.

A first food model is made by mixing agarose and gelatin in water to create an emulsion or a gel. By varying the amount of gelatin the size of the gelatin droplets can be changed to obtain a different microstructure. Two mixtures are considered: 1% agarose with 1% gelatin (Gel 1) and 1% agarose with 2.5% gelatin (Gel 2).

A second model food are the candy foams. These are produced by mixing fructose, dextrose agar-agar, albumin and water. Thanks to the presence of albumin air bubbles are formed resulting in a specific microstructure. Two mixtures have been considered in this study: One without dextrose (Foam 1) and one with dextrose (Foam 2).

The third model food considered in this study is a chocolate mousse which was created by mixing cold swelling starch with cocoa, sugar, oil and water. This results in a specific microstructure due to the presence of oil droplets and starch particles in the water matrix.

These different model foods are prepared in the lab and spatially resolved reflectance spectra are acquired on these. On each model food multiple SRS measurements are performed at different locations to access the variation in optical properties within a model food and to be able to compare this to the differences among different model foods. The optical properties of the different model foods are then estimated to extract chemical information from the absorption coefficient spectra and microstructure information from the scattering coefficient spectra.

RESULTS & DISCUSSION

Validation of the procedure for estimation of optical properties by solid phantoms

Figure 2 shows spatially-resolved reflectance profiles of a solid phantom \((\mu_s' = 20 \text{ cm}^{-1}, \mu_a = 0.14 \text{ cm}^{-1})\) in the range 450 – 1050 nm (high signal-to-noise regions). The unit vertical z-axis is relative reflectance calculated as the ratio of the dark-corrected intensity acquired for the sample by the dark-corrected intensity collected in an integrating sphere. In this way, the measured signals are compensated for dark noises, variations of light source intensity due to sensor sensitivity, differences in efficiencies of different pixels of the camera and differences in efficiencies of the detection fibers. Fiber 1 is the closest fiber and fiber 7 is the furthest one from the illuminating fiber. A clear decrease of the relative reflectance with increasing fiber number can be observed. This can be explained by the fact that light exiting the sample at a larger distance from the incident light beam has traveled a longer path through the sample and thus had more chance to be absorbed or scattered. A quite flat spectrum observed at each fiber position is a good indication that the black ink absorbs all wavelengths in the range 450 – 1050 nm.

![Spatially-resolved reflectance profiles of a solid phantom \((\mu_s' = 20 \text{ cm}^{-1}, \mu_a = 0.14 \text{ cm}^{-1})\)](image)

The optical properties of 16 solid phantoms estimated by the aforementioned fitting procedure are presented together with the actual optical properties in Figure 3 and Figure 4.
Figure 3. Fitted reduced scattering coefficients of 16 solid phantoms. Two levels of actual reduced scattering values are denoted by C ($\mu_s' = 15 \text{ cm}^{-1}$) and D ($\mu_s' = 20 \text{ cm}^{-1}$). At each scattering level there are 8 phantoms (denoted by numbers from 1 to 8) representing 8 actual absorption values.

Figure 4. Fitted absorption coefficients of 16 solid phantoms. The blue diagonal line represents the target line. The obtained results indicate quite good accuracy of the estimated optical properties for these solid phantoms which are considerably more scattering than absorbing ($\mu_s' \gg \mu_a$). In Figure 4, relatively bigger differences between the fitted values and the actual ones can be observed for the phantoms with higher absorption values. This can be explained by the fact that the diffusion approximation only holds for samples with considerably higher scattering than absorption ($\mu_s' \gg \mu_a$). When the absorption coefficient increased, but the scattering coefficient remains the same, larger errors can thus be expected. Another contributing factor could be the loss of a fraction of the diffusely reflected light which has not been captured by the detection fibers due to imperfect contact to the phantom surfaces, numerical aperture,...; which subsequently resulted in an over-estimation of the absorption values.

**Optical characterization of the model foods**

Figure 5 shows the estimated reduced scattering coefficients $\mu_s'$ for the different model foods. The standard deviations on the estimated reduced scattering coefficient $\mu_s'$ values are quite small compared to their mean values. This indicates that there were only minor variations in microstructure within each model food. A closer look at the estimated values for the reduced scattering coefficient $\mu_s'$ for the different model foods shows a logical correlation with the microstructures acquired by microscopy. The candy foams (Foam 1 and Foam 2) have the highest scattering coefficients, which can be explained by the strong scattering caused by the high concentration of air bubbles. The Chocolate Mousse has lower reduced scattering coefficients due to the low concentration of oil droplets and starch particles whose size ranges from 400 to 1100 nm in the water.
matrix. The lower difference in the refractive indices of water and oil or starch, compared to the difference in refractive index between water and air explains the higher scattering coefficient of the foams. For Gel 1 (1% gelatin) the estimated scattering coefficients are higher than those for Gel 2 (2.5% gelatin), which can be explained by the difference in their microstructures. Gel 1 has many small structures with similar size to that of the wavelength range considered (0.4 – 1.1 μm), causing strong light scattering. On the other hand, Gel 2 has less, larger structures which make that the number of interfaces where the light is refracted and reflected (scattered) is smaller for the same sample volume. This explains the lower light scattering by Gel 2. ‘Peaks’ of estimated reduced scattering coefficients of Gel 1 and Gel 2 appear unexpectedly around 970 nm probably due to violation of the assumption of much larger scattering than absorption in the regions with high water absorption.

Figure 5. (Upper) Fitted μ's of 5 model foods. The continuous and dotted lines represent the mean values and 95% confidence intervals of the fitted μ's, respectively. (Bottom) Corresponding microstructure of the model foods by microscopy (the scales in the microscopic images are 10 μm). Only high signal-to-noise regions are shown.

Figure 6. Fitted μa of 5 model foods. The continuous lines represent the mean values of the fitted μa. Only high signal-to-noise regions are shown.

A closer look at the absorption coefficient spectra shows that the absorption by these model foods in the wavelength range 400 – 1100 nm is rather limited except for Chocolate Mousse (Figure 6). Small water absorption peaks can be observed around 970 nm for both Foam 1 (without dextrose) and Foam 2 (with
dextrose). The broad peak before 750 nm for the Chocolate Mousse is expectedly attributed by chocolate pigments; which is in good agreement with the dark brown color of the Chocolate Mousse. It also noted that the estimated absorption coefficients below 600 nm of Gel 2 are bigger than those of Gel 1. This is also supported by the slightly darker color of Gel 2 compared to that of Gel 1 (Photos not shown here). The peaks of water absorption around 970 nm of Gel 1, Gel 2 and Chocolate Mousse are not observable probably due to the aforementioned violation of the assumption of much larger scattering than absorption at the basis of the diffusion approximation which may have led to overestimation of the reduced scattering coefficients and underestimation of the absorption coefficients in this region.

CONCLUSION

A spatially-resolved spectroscopy setup based on a fiber-optic probe was successfully elaborated in the lab and validated for the estimation of optical properties using solid phantoms with known optical properties. Several model foods with different designed microstructures were prepared and their optical properties were successfully estimated with this SRS setup. A logical correlation was found between the estimated reduced scattering coefficient spectra and the designed microstructures of these model foods, verified by light microscopy. The estimated absorption coefficients also showed good agreement with the designed ingredients. The obtained results clearly indicate the potential of spatially resolved spectroscopy methods for non-invasive food quality inspection and process monitoring in the food industry.

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